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UNIVERSITY OF ALBERTA

BIOAVAILABILITY IN ENVIRONMENTAL HEALTH RISK ASSESSMENT

by

WEIPING CHEN ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

DEPARTMENT OF HEALTH SERVICES ADMINISTRATION
AND COMMUNITY MEDICINE

EDMONTON, ALBERTA

Spring, 1994

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

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Abstract

Health risk assessment is a process whereby the likelihood of adverse human health effects arising from exposure to environmental contaminants is evaluated. The bioavailability factor is a measure fraction of total quantity of an environmental contaminants that an individual is exposed to that enters the bloodstream. This parameter can be important for quantitative health risk assessment as it is commonly used to calculate internal dose or adjust toxicity values (reference doses or slope factors). Currently, quantitative information about bioavailability for most contaminants is limited. Thus, an understanding of bioavailability study is important to achieving a sound scientific basis for quantitative health risk assessment and to improve the development of environmental policy and regulatory decisions.

In this thesis, the basic concept of bioavailability, mechanisms of absorption of contaminants by three main exposure routes and common or specific experimental methods for bioavailability study are discussed. The roles and applications of bioavailability in quantitative risk assessment are explored. The toxicological basis, quality of experimental methods and interpretation of bioavailability are evaluated for about 30 selected environmental contaminants found at the former industrial sites in Alberta. Based on a critical review of the scientific literature, the range of bioavailability factors for those contaminants has been determined. Relevant exposure routes for the contaminants are discussed and some needs for further research in bioavailability are presented.

The bioavailability factor in some cases can contribute to achieving improved accuracy in quantitative health risk assessment. However, in general, the bioavailability factor is an approximate parameter because a variety of factors may influence bioavailability via various exposure routes. In practice, the characteristics of the bioavailability estimates that are selected for calculation of internal dose or adjustment of toxicity values should be close to those from the situation under study in order to minimize the uncertainties. The logic for reliably applying bioavailability factors in health risk assessment is summarized.

ACKNOWLEDGEMENTS

The author is deeply grateful to her supervisor, Dr. S. E. Hrudey, for his support, supervision and participation throughout the course of this study.

The funding for this study was provided by the National Science and Engineering Research Council and by an Imperial Oil University Research Grant. This support is most gratefully acknowledged.

The author would like to thank Nola Low for her support.

Finally, the author wishes to thank her husband, Qishi for his encouragement and support, and her son, Edmond, for his patience and understanding.

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Glossary of Terms

Absolute bioavailability – the fraction or percentage of external dose that is present in the systemic circulation, that is, the ratio of internal dose to external dose. This is called the *bioavailability factor*.

Absorbed dose – see internal dose.

Absorption – a process whereby xenobiotics cross body boundaries and reach the bloodstream.

Absorption barrier – any biological barrier of body where various substances can cross to reach the bloodstream. The most common absorption barriers include alveolar wall (*air-blood barrier*), gut wall (*gut-blood barrier*) and skin.

Active transport – a process whereby a xenobiotic that forms a complex with a carrier on one side of cell membrane crosses absorption barriers against concentration or electrochemical gradient, requiring expenditure of energy.

Adherence fraction for soil – the proportion of soil contacted that will adhere to the skin.

Administered dose – the amount of a xenobiotic that is actually inhaled, ingested or applied on the skin. This dose, sometimes, is called *potential dose* in the literature.

Aerosol – a relatively time-stable suspension of solid and liquid particles in the atmosphere, with a range of 0.001-100 μm in diameter. Aerosols often refer to various aerodisperse system which reflect their origin, physical state, and range of particle size, as well as the performance characteristics of the analyzer. Aerosols include *dust, smoke, fume, mist, fog, cloud, smog, fine particles, coarse particles, total suspended particles, primary aerosols, secondary aerosols, and inhalable particulate matter*.

Air-blood barrier – see absorption barrier.

Alveolar ventilation – the tidal volume minus the volume of the dead space.

Applied dose – the amount of a xenobiotic contact with the surface of primary absorption sites (e.g. alveoli, gut wall and skin).

Area under blood concentration-time curve (AUC) – the concentration of a xenobiotic in blood (plasma or serum) integrated over time.

Average daily dose – the dose per unit body weight over an average time, including the average daily internal dose and the average daily external dose.

Bioavailability – the extent and rate of absorption for a xenobiotic (a foreign substance) which enters the systemic circulation in the unaltered (parent) form from the applied exposure site.

Bioavailability factor – see absolute bioavailability .

Bioavailable dose – see internal dose.

Biologically effective dose – the delivered mass of active toxic species at the sites of action.

Blood-air partition coefficient – a constant of the solubility ratio of a xenobiotic between the blood and air.

Cloud – any free aerosol system with a definite size and form, without regard to particle size.

Coarse particles – aerodynamic diameters of aerosols between 2.5-15 µm, which are produced by mechanical process, and collected by dichotomous sample.

Diffusion coefficient – a constant of the ratio of water solubility (S) of a xenobiotic and molecular weight (MW), as: $\frac{S}{MW^{1/2}}$.

Deposition coefficient – a fraction of total inhaled gas or vapor that deposits on the airway wall of respiratory tract. It is also called *total intake*.

Dissolution – water soluble constituents are eluted from the matrix of particles.

Distribution – a process whereby the absorbed xenobiotics are transported by the bloodstream to various organs and tissues.

Dose – the total mass of a contaminant that actually crosses the body layer, absorbed or deposited into the body, and reaches the target sites over a given period.

Dose-response relationship – a correlative relationship between the magnitude of various doses and intensity of toxic response following exposure.

Dust – dry solid particles dispersed in the air formed by grinding, milling, crushing, or blasting without regard to particle size.

Enterohepatic circulation – a process whereby xenobiotics and their metabolites entering the intestine with biliary excretion may be reabsorbed into the portal blood and returned to the liver.

Excretion – a process whereby xenobiotics are eliminated from the body by several routes, such as urine, feces and lungs.

Exposure – the environmental contaminants experienced by an individual or a population.

Exposure dose – the total mass of a xenobiotic in the environmental media that an individual or population is exposed to over time.

External dose – the total mass of a xenobiotic at the applied site available for absorption. External dose in the literature generally implies a variety of doses, such as *applied dose, administered dose,* and *exposure dose,* depending on the designs and analytical methods involved in experiments.

Facilitated diffusion – a process whereby a xenobiotic that forms a complex with a carrier on one side of cell membrane crosses absorption barriers but not against concentration or electrochemical gradient and not requiring expenditure of energy.

Filtration – a process whereby a xenobiotic penetrates the absorption barriers following bulk water flux without expenditure of energy.

Fine particles – aerodynamic diameters of aerosols between 0-2.5 μm, which are formed by condensation and coagulation, and collected by dichotomous sampler.

First-pass effect – this effect refers that some xenobiotics will be metabolized at the applied sites (the airway wall, the gut wall and the skin) or during passage through the liver from the portal vein prior to the systemic circulation. It is also called presystemic metabolism.

Fogs – liquid droplets, with visibility.

Fumes – condensation aerosols consisting of small liquid and solid particles formed by condensation of vapors, combustion, and sublimation, with a range of 0.01-1.0 µm in diameter.

Gut-blood barrier – see absorption barrier.

Health risk assessment – a process that seeks to estimate the adverse health consequence of a specified set of conditions. The assessment process includes: (1) hazard identification; (2) dose-response assessment; (3) exposure assessment; and (4) risk characterization.

Hepatic clearance – the rate of total body clearance relevant to the liver.

Hygroscopicity – some water-soluble aerosols will grow in moist air until they reach equilibrium, e.g. sea salt, tobacco smokes and sulfuric acid droplets.

Inertial impaction – this process means that particulates (ranging from 5 to 30 µm) continue to travel along their original direction because of their inertia despite changes in the airstream direction.

Inhalability – see inhalable fraction.

Inhalable fraction – the portion of the total mass of airborne aerosols that actually cross the nose and mouth. It is also called *intake efficiency* or *inhalability*.

Inhalable particulate matter (IPM) – aerosols less than 10-15 µm in diameter (IPM_{10} or IPM_{15}) that may be inhaled into the respiratory tract by humans.

Interception – this process occurs at bifurcations of bronchial trees only for long fibrous particulates (e.g. asbestos).

Intake efficiency – see inhalable fraction.

Internal dose – the total mass of a xenobiotic that is actually absorbed into the systemic circulation. Internal dose, sometimes, is called the "absorbed dose" or "bioavailable dose".

Lifetime average daily dose –the dose per unit body weight over an average lifetime, including the lifetime average daily internal dose and the lifetime average daily external dose.

Mass median aerodynamic diameter (MMAD) – this measure reflects the reality that sampling methods used to characterize particle size distribution depend upon the aerodynamic properties rather than absolute size of particles. The aerosol size parameters are aerodynamic diameters for aerosols ≥ 0.5 μm.

Mass median diffusive diameter – a diffusion diameter for aerosols < 0.5 μm that reflects the diffusion properties of aerosols.

Mechanical translocation – a process whereby a xenobiotic is removed from the respiratory tract by various physical processes, such as sneezing, wiping, blowing, coughing, mucociliary transport and phagocytosis.

Metabolism – a process whereby the absorbed xenobiotics are converted from one form to other forms by enzymatic systems in living organisms.

Mists – invisible liquid droplets formed by condensation of vapor, having diameter greater than 10 μm.

Passive transport – a process whereby a xenobiotic crosses absorption barriers without expenditure of energy, including *simple diffusion* and *filtration*.

Peak height (C_{max}) – the measured values of peak concentration of a xenobiotic in the blood. This is one of parameters for estimating the rate of absorption of a xenobiotic.

Peak time (t_{max}) – time needed to reach the peak concentration. This is one of parameters for estimating the rate of absorption of a xenobiotic.

Permeability – the rate of diffusion of a xenobiotic through each unit area of the barrier for a unit concentration difference.

Persorption – a process whereby large molecules, e.g. starch granules and diatomaceous earth particles, may enter the intercellular space from a gap at the tip of the villi between two cells which are sloughed off.

Phagocytosis – a process whereby a solid xenobiotic is removed by engulfing of cells.

Pinocytosis – a process whereby a liquid xenobiotic is removed by engulfing of cells.

Portal circulation – portal venous blood between the gut and liver.

Potential dose – see administered dose.

Presystemic metabolism – see first-pass effect.

Primary aerosols – dispersion aerosols, directly emitting into the air without change of shape in the air.

Rate-limiting step – the step of the slowest rate of constant in the absorption processes.

Relative bioavailability – When intravenous administration is not feasible (such as solution problem), the bioavailability factor may be estimated from relative bioavailability. It is determined by a comparison of the extent of absorption among several doses of the same xenobiotic, or two or more forms of the same xenobiotic, or different vehicles (food, soil and water) of the same xenobiotic, with the same subject. The extent of absorption for the test material (f_2) is compared to that for the standard material (f_1), as: relative bioavailability $= \frac{f_2}{f_1}$.

Secondary aerosols – dispersion aerosols formed by chemical reactions.

Sedimentation – a process whereby the small particles (1 to 5 μm) settle in the small bronchi, bronchioles and the alveolar space by gravitational force.

Smog – highly visible mixtures of particles and gases associated with urban aerosol formation (e.g. automobile exhaust).

Simple diffusion – a process whereby a xenobiotic penetrates the absorption barriers followed by Fick's law without expenditure of energy.

Smokes – visible, dispersion aerosols containing a variety of solids, liquids, and gases formed by condensation of supersaturated vapors, with a range of 0.1-10 μm in diameter.

Systemic circulation – both the arterial and venous blood except the portal blood.

Total intake – see deposition coefficient.

Total suspended particles (TSP) – aerosols collected by the high-volume sampler.

Toxicokinetics – the study of kinetics of xenobiotics, including quantitation and determination of the time course of disposition (absorption, distribution, metabolism and excretion) of xenobiotics in the organism.

Ventilation/perfusion ratio (V_A/Q) – the ratio of ventilation (V_A) to blood flow (Q) remains constant although the ventilation or blood flow changes with time.

Ventilation volume – the volume of air inspired or expired during different inhalation stages, including the tidal volume, inspiratory reserve volume, expiratory reserve volume, residual volume, inspiratory capacity, functional residual capacity, vital capacity, and total lung capacity. *Tidal volume*: the volume of air inspired or expired during restful breathing, about 500 ml for an adult health man. Minute volume can be calculated by the product of the tidal volume and the breathing frequency (15 times/min). *Inspiratory reserve volume*: the extra volume of air that can be inspired by forceful inspiration, about 3000 ml. *Expiratory reserve volume*: the extra volume of air that can be expired by forceful expiration, about 1100 ml. *Residual volume*: the volume of air remaining in the respiratory tract following forceful expiration, about 1200 ml. *Inspiratory capacity*: the sum of the tidal volume and inspiratory reserve volume, about 3500 ml. *Functional residual capacity*: the sum of the expiratory reserve volume and residual volume, about 2300 ml.*Vital capacity*: the sum of the inspiratory reserve volume, expiratory reserve volume, and tidal volume, about 4600 ml.*Total lung capacity*: the sum of the vital capacity and residual volume, about 5800 ml.

1. Introduction

Human health risk assessment is a process that seeks to estimate the adverse health consequence of a specified set of conditions. The assessment process includes: (1) hazard identification; (2) dose-response assessment; (3) exposure assessment; and (4) risk characterization (USEPA 1989).

Hazard identification is the process of identification of xenobiotics that are harmful to human health, and a qualitative evaluation of their character and importance.

In *dose-response assessment*, the quantitative relationship between any dose (external dose, internal dose, or biologically effective dose) and the likelihood of an adverse health effect is described. The toxicity values are usually obtained from animal experiments by administration of xenobiotics at external doses (or less commonly with internal doses) involving different environmental media through various exposure routes. The criteria values determined are expressed as reference dose (RfD) for xenobiotics that produce noncarcinogenic effects and a potency (slope factor) used to calculate a risk specific dose (RsD) for xenobiotics that induce carcinogenic effects over a lifetime.

Exposure assessment provides qualitative insight and/or quantitative data on the magnitude of human exposure to xenobiotics, exposure duration and frequency, sources of exposure, routes of exposure, characteristics (size, nature and class) of the potentially exposed population. This process evaluates the human exposure pathways (source of contamination, environmental media and transport, point of exposure, and receptor population) and the calculation of average daily doses or lifetime average daily doses (external doses and internal doses).

Parameters needed for calculation of these doses via the main exposure routes are listed in Table 1-1. The environmental parameters (concentrations of xenobiotics in various media) may be obtained by direct measurement from environmental monitoring or indirectly by mathematical modeling. The physiological parameters (inhalation rate, ingestion rate, skin surface area and body weight) may be obtained by searching in the literature, handbooks and some databases. Bioavailability factor and some other factors (deposition fraction and dissolution fraction) may be obtained from animal experiments (or human experiments in the past).

Table 1-1 Parameters needed for Calculation of External Dose and Internal Dose

Inhalation and Ingestion Exposure Routes	Dermal Exposure Route
$ADD_{int} = \frac{\overline{C}\ \overline{IR}\ ED\ OF\ BF}{BW\ AT}$ $ADD_{ext} = \frac{\overline{C}\ \overline{IR}\ ED\ OF}{BW\ AT}$	*For waterborne contaminants and vapors* $ADD_{int} = \frac{\overline{C}\ P\ \overline{SA}\ ED}{BW\ AT}$ *For soil-bound contaminants* $ADD_{int} = \frac{\overline{C}\ Fadh\ \overline{SA}\ ED\ BF}{BW\ AT}$ $ADD_{ext} = \frac{\overline{C}\ Fadh\ \overline{SA}\ ED}{BW\ AT}$
ADD = average daily dose $\overline{C}$ = average concentrations of a xenobiotic in air (mass/m^3), in drinking water (mass/L), in food (mass/g), and in soil (mass/kg)	
$\overline{IR}$ = average inhalation rate (m^3/day), or ingestion rate for total or tap water (L/day), soil (mass/day), and food (mass/day)	P = dermal permeability (cm/h)
ED = exposure duration (days or years)	F_{adh} = adherence fraction for soil (mass/cm^2)
BW = body weight (kg)	$\overline{SA}$ = average exposed skin surface area (cm^2)
AT = average exposure time period (days or years); For calculating lifetime average daily does, LA (lifetime average exposure time period) replaces AT	BF = dermal bioavailability factor (unitless)
BF = inhalation bioavailability factor, or ingestion bioavailability factor (unitless)	
OF = other factors, such as *deposition fraction* that is used to adjust to external doses for aerosols, or to adjust internal dose when the respiratory bioavailability factor is based on the deposited dose of an aerosol; *dissolution fraction* that is used to adjust internal doses when soil-bound xenobiotics will dissolve in the body fluid within the respiratory or gastrointestinal tract before absorption	

Adapted from: USEPA 1992a with a modification

Risk characterization is a description of the nature and extent of human risk by comparison of all the results from the foregoing steps with a discussion of attendent uncertainties and analytical assumptions.

For risk assessment to be valid, estimates of dose from an exposure assessment should be expressed in a manner that can be compared with toxicity values. Within the overall context of risk assessment, the bioavailability factor is primarily used to adjust an external dose to an internal dose in exposure assessment, and occasionally to adjust the toxicity values in dose-response assessment when the exposure media from the environment differ from those from the toxicity studies. For this purpose, bioavailability is defined as the availability of foreign substances to reach the human systemic circulation. Frequently, this value is less than the external exposure dose because of a number of factors involved in the absorption process for various exposure routes. The most common routes of entry for environmental contaminants into humans are inhalation, ingestion, and dermal absorption.

Currently, the validity and reliability of quantitative risk assessment are often restricted by the absence of quantitative data in relation to underlying physicochemical, environmental and biological processes. Determination of bioavailability is one critical issue that can improve the accuracy of exposure assessment and prediction of health effects. Ultimately, better knowledge of bioavailability will lead to more accurate evaluation of the potential risk for humans arising from exposure to environmental contaminants, and improve the development of environmental policy and regulatory decisions.

In order to study bioavailability, the structure, function, absorption mechanism, and variations in all absorption processes by major exposure routes must be understood. Interpretation of data from bioavailability studies requires an understanding of the design, measurement, strengths and limitations of experimental methods.

The basic knowledge on bioavailability is briefly reviewed in this thesis. The application of bioavailability in quantitative risk assessment is demonstrated. Many factors that may influence bioavailability are presented. The absorption mechanisms via inhalation, ingestion, and dermal exposure are discussed. Experimental methods used in bioavailability studies, including *in vivo* and *in vitro*, are identified and described.

The roles and applications of bioavailability for various waste components at a number of former industrial sites in Alberta (HELP sites, i.e. the HELP End Landfill Pollution project of Alberta Environment), are explored, specifically for former wood preserving sites.

These waste components can be classified into three groups: chromated copper arsenate (CCA)-based mixtures, pentachlorophenol(PCP)-based mixtures, and creosote-based mixtures. Most chemicals presented in these groups are harmful for human health. Some are considered as carcinogens and possible or probable carcinogens for humans and animals. For the purpose of quantitative risk assessment, it is crucial to determine how and how much the chemicals enter the human body to exert toxic effects.

The objective of this study is to derive bioavailability data for the chemicals identified at the contaminated sites based on the available information in the literature. However, studies on bioavailability for most environmental contaminants are incomplete. Some level of uncertainty is involved in the use of bioavailability for risk assessment. Meanwhile, a better understanding of the mechanisms of toxicity and carcinogenicity for xenobiotics will lead to better uses of the bioavailability concept for quantitative risk assessment.

In addition to the determination of bioavailability, in this thesis, the possible exposure routes for the general population living near the contaminated sites are identified. Uncertainty related to bioavailability is analyzed. Information on the needs for further research is provided.

2. Fundamental Concept of Bioavailability

2.1 Definition of Bioavailability

Recently, many basic pharmacokinetic concepts have been applied for toxicology. The term "bioavailability" originates from one of the pharmacokinetic concepts. Bioavailability is extensively used to describe the extent and rate of absorption for a xenobiotic (a foreign substance) which enters the systemic circulation in the unaltered (parent) form from the applied exposure site. Recently, there have been substantial advances in modelling the complete behavior of xenobiotics in the body. These models, called physiologically based-pharmacokinetic (PBPK) models, include bioavailability concept as one small component of the total disposition of the xenobiotic. In principle, there are three general issues involved in the bioavailability concept: the systemic circulation, the extent of bioavailability, and the rate of bioavailability.

Systemic Circulation

"Systemic circulation", sometimes called the bloodstream, refers to both the arterial and venous blood except the portal blood (Ritschel 1972). A xenobiotic administered via inhalation and dermal route is directly absorbed to the systemic circulation. But a xenobiotic in the gastrointestinal tract initially reaches the portal vein, and then passes through the liver into the systemic circulation. There are three reasons for applying this term: (1) a xenobiotic only in the systemic circulation is available for reaching all the target sites (other than the liver during ingestion exposure) in the body; (2) in most experiments, the practical site for the measurement of absorbed dose is in the systemic circulation. Also, the amount of a xenobiotic in the portal blood is difficult to measure for humans; (3) the dose-response relationship of a xenobiotic is often closely correlated with the concentrations of the xenobiotic in the systemic circulation. Currently, the use of bioavailability referring to the proportion of xenobiotic reaching the systemic circulation is more prevalent. Thus, this concept has also been termed the "systemic availability".

Extent of Bioavailability

The extent of bioavailability for a xenobiotic is determined by either absolute bioavailability or relative bioavailability.

Absolute bioavailability. Absolute bioavailability is usually determined by the measure of the fraction or percentage of external dose that is present in the systemic circulation, that is, the ratio of internal dose to external dose. This is called the **bioavailability factor** (BF). The equation is given by

$$BF = \frac{\text{internal dose}}{\text{external dose}} (x100) \qquad (2\text{-}1)$$

where **external dose** represents the total mass of a xenobiotic at the applied site available for absorption, and **internal dose** is the total mass of a xenobiotic that is actually absorbed into the systemic circulation.

Internal dose, sometimes, is called the "absorbed dose" or "bioavailable dose". For dermal and inhalation exposure, the dose refers to the degree of a xenobiotic that directly crosses the barrier at the absorption site, i.e. the skin and the membrane of the alveoli in the lung, respectively. In the case of oral exposure, a xenobiotic penetrates the gut wall into the portal blood. Then, the xenobiotic is extracted by the liver where the xenobiotic may undergo presystemic metabolism. If metabolized, the total mass of the parent xenobiotic will decrease before entering the systemic circulation. As a result, the internal dose is less than the dose in the portal blood. This phenomenon is described as the "hepatic first-pass effect" or "hepatic presystemic metabolism".

The internal dose for most xenobiotics only refers to the presence of the parent compounds, not metabolites, in the systemic circulation. With the advances in understanding the mechanisms of toxicity, particularly carcinogenicity, for many environmental contaminants, limiting the internal dose to the unaltered form will not fully characterize the dose-response relationship for some xenobiotics. For instance, the carcinogenicity of benzene is considered to be related to its three hepatic metabolites. Therefore, the identification and quantification of its metabolites are critical for assessing the benzene cancer risk. By extension, in such a case, the internal dose should include the amount of the metabolites of benzene in the systemic circulation.

External dose in the literature generally implies a variety of doses, such as applied dose, administered dose, and exposure dose, depending on the designs and analytical methods involved in experiments. Theoretically, **applied dose** that refers to the amount of a xenobiotic contact with the surface of primary absorption sites (e.g. alveoli, gut wall and skin) is an ideal parameter for accurately assessing the bioavailability factor. In practice, applied dose for most xenobiotics is difficult to measure with *in vivo* experiments for inhalation, ingestion and dermal exposure (other than dermal contact with vapors and waterborne contaminants). In most cases, administered dose (sometimes, exchangeable for labeled dose or potential dose or exposure dose) is frequently employed to describe the bioavailability factor. **Administered dose** is the amount of a xenobiotic that is actually inhaled, ingested or applied on the skin. **Exposure dose** is commonly defined as the total mass of a xenobiotic in the environmental media that an individual or population is exposed to over time (USEPA 1992a, Lioy 1990a, NRC 1991). Administered dose may be estimated from exposure dose if contact rates (e.g. inhalation rate and ingestion rate) are known.

Additionally, there may exist a great difference between the **administered dose** and the **applied dose** after inhalation of a slightly water-soluble aerosol. The xenobiotic administered by inhalation will be initially eliminated via exhalation and mechanical clearance by which the inhaled xenobiotic is swallowed into the gut or removed to pulmonary lymph nodes. In such a case, only a small fraction of the inhaled xenobiotic that is deposited but not mechanically cleared, i.e. the applied dose, will be available for absorption.

Generally, **external dose** is used as **administered dose** in most bioavailability experiments.

There are two basic *in vivo* methods for evaluating the bioavailability factor in humans and in animals (Wagner 1969, 1972, 1977). One is the measurement of the blood levels of a parent xenobiotic over time. The other is the measurement of the total mass of the unaltered form in excreta, such as urine, feces and expired air. In such studies, the blood levels or the cumulative excreta data for a xenobiotic following administration by extravascular routes (e.g. inhalation, ingestion and dermal) are compared to those after intravenous administration. Intravenous dose provides a reference dose, a bioavailability factor equal to one. The other useful *in vivo* method, called the "mass-balance technique", is applicable in animals. There are various specific *in vivo* and *in vitro* methods available for determining the extent of bioavailability for each specific exposure route.

Relative bioavailability. When intravenous administration is not feasible (such as solution problem), the bioavailability factor may be estimated from relative bioavailability. Relative bioavailability (BF_R) is determined by a comparison of the extent of absorption among several doses of the same xenobiotic, or two or more forms of the same xenobiotic, or different vehicles (food, soil and water) of the same xenobiotic, with the same subject (Labaune

1989). For instance, when the pure form of a xenobiotic is used as a standard material, any other form of the xenobiotic is used as a test material. The extent of absorption for the test material (f_2) is compared to that for the standard material (f_1), as:

$$BF_R = \frac{f_2}{f_1} (x100) \qquad (2\text{-}2)$$

If absolute bioavailability for the standard material is known (BF_1), absolute bioavailability for the test material (BF_2) can be calculated by:

$$BF_2 = BF_R \times BF_1 \qquad (2\text{-}3)$$

Rate of Absorption

The rate of absorption is another important issue in the assessment of bioavailability. With the comparison of two xenobiotics, the concentrations in the systemic circulation may be similar but the rate of absorption from different doses may differ. A slowly absorbed xenobiotic may require a higher external dose to reach the same blood concentration in a given exposure time and to exert a toxic effect equivalent to a more rapidly absorbed xenobiotic.

There are a number of mathematical models for determining the rate of absorption, such as the one-compartment model (Wagner-Nelson method), multicompartment model (Loo-Riegelman method), noncompartment model, statistical moment theory, convolution and deconvolution model (Gibaldi and Perrier 1982). Typically, the measured values of peak concentration of a xenobiotic in the blood (C_{max}) and time needed to reach the peak concentration (t_{max}) can be employed to roughly estimate the rate of absorption. The value of t_{max} is inversely related to absorption rate.

2.2 Bioavailability in Health Risk Assessment

Health risk assessment can be considered to consist of (1) hazard identification; (2) dose-response assessment; (3) exposure assessment; and (4) risk characterization (NAS 1983). Human health risk assessment is related to the evaluation of the likelihood of adverse human health effects caused by environmental contaminants.

The exposure assessment is both an important and complex step. The process involves the characterization of exposure setting, identification of exposure pathways, and quantification of exposure (USEPA 1989a).

With regard to health outcome, the exposure and dose are basic determinants of the toxic responses. The dose-response relationship which is the core of health risk assessment must rely upon an accurate estimation of the exposure and effective dose.

Exposure reflects the environmental contaminants experienced by an individual or a population. The extent of exposure (E) is the total concentration of a contaminant at the exposure point over time (Lioy 1990a, NRC 1991):

$$E = \int_{t_1}^{t_2} C(t)\, dt \tag{2-4}$$

where C(t) is the concentration at the point of contact as a function of time, and t_2-t_1 represents the exposure duration. Exposure can be readily measured in most cases, but, by itself, offers little insight into health consequences.

Dose is the total mass of a contaminant that actually crosses the body layer, absorbed or deposited into the body, and reaches the target sites over a given period (NRC 1991). The general equation of dose (D) is as (Lioy 1990a):

$$D = \int_{t_1}^{t_2} D(t)\, dt \tag{2-5}$$

The dose has various definitions in the application of risk assessment.

Biologically Effective Dose

The delivered mass of active toxic species at the sites of action is usually defined as the **biologically effective dose**. Evaluation of this dose (D_{bio}) is based on cell repair or damage, metabolism, excretion, and chemical assimilation (Lioy 1990a):

$$\int D_{bio}(t)\, dt = \int PF\ BF\ IR\ C(t)\, dt \tag{2-6}$$

where PF represents pharmacokinetic fraction (transport, metabolism, and excretion), BF is the bioavailability factor, and IR refers to the contact rate (inhalation rate and ingestion rate).

Theoretically, the biologically effective dose is a critical parameter for evaluating the dose-response relationship. It may be estimated by biological monitoring (measures of DNA and protein adducts in the blood, cells and tissues) and mathematical modeling based on the physicochemical properties of chemicals, and physiological and pharmacokinetic parameters available (NRC 1989, 1991). However, routine use of this dose for quantitative risk assessment may not be practical, because:

- This dose is difficult to be measured in the human body and in most animals because of limitations with the experimental designs and analytical techniques.
- The data base (several pharmacokinetic parameters) for this dose to be used in mathematical modeling is rarely available for most environmental contaminants.
- This dose cannot be traced directly back to determine the original source of environmental contaminants, or to identify the exposure routes because any xenobiotic-DNA (or protein) adducts are not specific for any particular exposure source or route.

Internal Dose and Average Daily Internal Dose

Studies for determining the dose-response relationship have shown that the internal dose is closely related to the occurrence of toxic effects for many xenobiotics. Quantification of internal dose may also involve the calculation of the average daily internal dose defined as the internal dose per unit body weight over an average time.

The bioavailability factor has been widely used to evaluate the internal dose and the average daily internal dose in quantitative risk assessment. The internal dose (D_{int}) is expressed by (Lioy 1990a, USEPA 1992a):

$$\int D_{int}(t)\,dt = \int BF\ IR\ C(t)\,dt \qquad (2\text{-}7)$$

If C and IR can be approximated by average values, D_{int} can be written by

$$D_{int} = \overline{C}\ \overline{IR}\ ED\ BF \qquad (2\text{-}8)$$

where $\overline{C}$ is the average concentration of an environmental contaminant, $\overline{IR}$ is the average value of contact rate, and ED is the total exposure duration.

The average daily internal dose (ADD_{int}) is

$$ADD_{int} = \frac{\overline{C}\,\overline{IR}\,ED\,BF}{BW\,AT} \tag{2-9}$$

where BW is body weight, in units of kg, and AT is the time period over which the dose is to be averaged, in units of days.

For the dermal exposure route, waterborne contaminants or vapors in contact with the skin can directly penetrate the skin barrier into the systemic circulation. The internal dose is dependent on the concentration of contaminants in water, the partial pressure of vapors, the dermal permeability, and the surface area exposed. The internal dose is given by (USEPA 1992a):

$$\int D_{int}(t)\,dt = \int_{t_1}^{t_2} C(t)\,P\,SA(t)\,dt \tag{2-10}$$

and

$$D_{int} = \overline{C}\,P\,\overline{SA}\,ED \tag{2-11}$$

where C(t) is the concentration of a contaminant in water (mg/L) or air ($\mu g/m^3$) in contact with the skin over time and $\overline{C}$ is the average concentration value; P represents the dermal permeability coefficient of chemicals (cm/h); SA refers to the surface area of skin exposed over time (cm^2) and $\overline{SA}$ is the average value. The average daily internal dose can be expressed as (USEPA 1992a):

$$ADD_{int} = \frac{\overline{C}\,P\,\overline{SA}\;ED}{BW\,AT} \tag{2-12}$$

For soil-bound contaminants, the internal dose can be determined from the contaminant concentration and the amount of carrier medium that is the product of the fraction of soil adhering to the skin, the surface area exposed, exposure duration and bioavailability factor (USEPA 1992a):

$$D_{int} = \overline{C}\,M_{medium} = \overline{C}\,F_{adh}\,\overline{SA}\,ED\;\;BF \tag{2-13}$$

where $\overline{C}$ is the average soil-bound contaminant concentration (mg/kg); M_{medium} refers to the mass of soil applied; F_{adh} represents the adherence fraction for soil, the proportion of soil contacted that will adhere to the skin. This algorithm does not explicitly deal with the reality that contaminants bound to the uneven surface of soil particles will be not uniformly in contact with the skin and will not all be available for transfer across the skin barrier.

ADD_{int} can be calculated by (USEPA 1992a):

$$ADD_{int} = \frac{\overline{C}\, M_{medium}\, BF}{BW\ AT} = \frac{\overline{C}\, F_{adh}\, \overline{SA}\, ED\, BF}{BW\ AT} \qquad (2\text{-}14)$$

A number of factors must be considered in judging the values of estimating bioavailability factors for the purpose of quantitative risk assessment. These include:

- The internal dose will be more specific for quantitative risk assessment than the external dose if toxicity values for a xenobiotic is based on the internal dose. This dose can provide a relatively identifiable relationship to the toxicity at the target sites.
- The internal dose may be estimated by knowing a few key environmental, physiological and pharmacokinetic parameters.
- The internal dose will reduce uncertainty in the risk assessment provided that the bioavailability factor is reliably estimated from experimental exposures.
- The dose can be traced directly to determine the original source of environmental contaminants, or to identify the exposure sites of receptors because of knowing the quantity of environmental contaminants and exposure pathways.

External Dose and Average Daily External Dose

Quantitative assessment of health risk for groups or subgroups of populations is an important part in health risk assessment. In practice, it is easier to estimate the external dose rather than the internal dose for populations. The equations for evaluating the external dose (D_{ext}) and the average daily external dose (ADD_{ext}) are (USEPA 1992a):

For inhalation and ingestion routes

$$D_{ext} = \overline{C}\, \overline{IR}\, ED \qquad (2\text{-}15)$$

and

$$ADD_{ext} = \frac{\overline{C}\,\overline{IR}\,ED}{BW\ AT} \tag{2-16}$$

For dermal contact with the soil-bound contaminants

$$D_{ext} = \overline{C}\,M_{medium} = \overline{C}\,F_{adh}\,\overline{SA}\ ED \tag{2-17}$$

and

$$ADD_{ext} = \frac{\overline{C}\,M_{medium}}{BW\ AT} = \frac{\overline{C}\,F_{adh}\,\overline{SA}\,ED}{BW\ AT} \tag{2-18}$$

These quantitative external dose estimates rarely reflect the most direct input to the dose-response relationship for many environmental contaminants. Using the external dose inevitably contributes to uncertainties in the assessment of health risk. For example, following inhalation of aerosols, the inhaled dose that reaches the surface of alveoli (before entering the systemic circulation) is reduced because of deposition, clearance and retention in other parts of the respiratory system. Consequently, the internal dose in the systemic circulation is considerably less than the external dose. Using the external dose in quantitative risk assessment effectively assumes that the bioavailability factor is equal to one.

Currently, many quantitative risk assessment protocols focus on estimation or determination of external dose and the average daily external dose. Reasons for this circumstance include:

- A reliable bioavailability factor has not been estimated in a form that is useful for risk assessment for most environmental contaminants.
- The dose-response relationship for many xenobiotics has been estimated by using only the external dose.
- Estimation of external dose only requires several environmental and intake rate parameters that are easily obtained.
- External dose can be traced directly to determine the original source of environmental contaminants, or to identify the exposure sites of receptors.

2.3 Summary

In order to identify whether the exposure doses from the environment exhibits a potential health risk for humans, the exposure doses can be compared to the dose that cause the toxic effects. Estimation of daily doses (average or lifetime) is commonly used for this purpose. However, various doses may be applied for quantitative risk assessment (Figure 2-1).

The parameter that should most accurately reflect the dose-response relationship is the biologically effective dose. But, the use of this dose, is generally not feasible because of the lack of biological measurements and required to provide the basis for the mathematical modeling.

The most common parameter is the external dose because it is readily determined through relatively simple approaches (direct measurement, biological monitoring and mathematical modeling). Nevertheless, the use of this dose is involved in a high level of uncertainty.

Between the ideal of the biologically effective dose and external dose is the internal dose that is generally well correlated with toxic responses. Bioavailability, the extent and rate of xenobiotic absorption, determines the internal dose and thereby contributes to quantitative risk assessment.

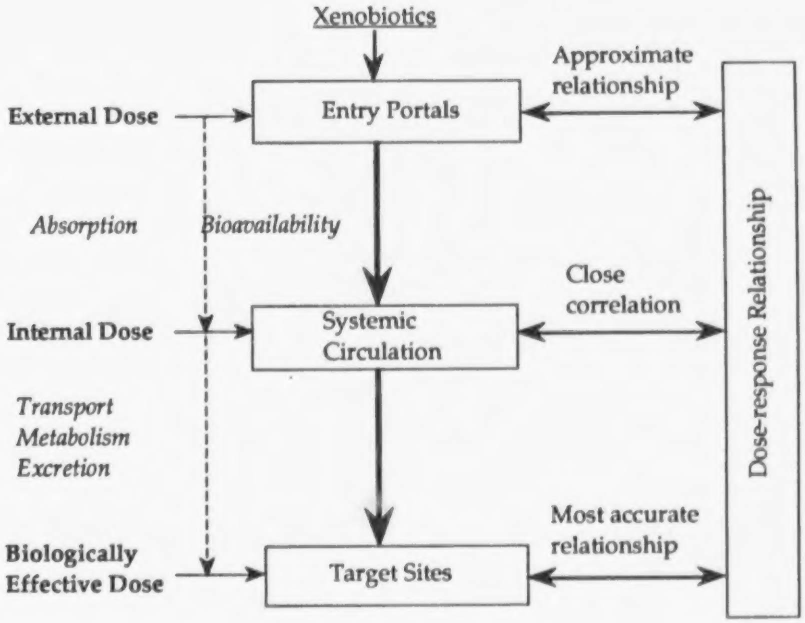
Xenobiotics
Approximate relationship
External Dose
Entry Portals
Absorption
Bioavailability
Close correlation
Internal Dose
Systemic Circulation
Dose-response Relationship
Transport
Metabolism
Excretion
Most accurate relationship
Biologically Effective Dose
Target Sites

Figure 2-1 Relationship of various doses, their role and application in quantitative risk assessment

3. Mechanism of Absorption

Xenobiotics must pass through one or more biological membranes during absorption. The biological barriers may consist of a single layer of the cell membrane (such as alveolar wall) or multiple layers of those membranes (such as skin). Cell membranes have similar structure and composition, which has been described as a "mayonnaise sandwich". Within the cell membrane, proteins are inserted in the lipid bilayer. The lipid composition varies from cell to cell, including various phospholipids, fatty acid, cholesterol and other steroids.

Transport of xenobiotics within an organism can be classified into (1) passive transport, including simple diffusion and filtration; and (2) special transport, including active transport, facilitated diffusion, phagocytosis, pinocytosis and persorption. The mechanism of absorption of xenobiotics via inhalation, ingestion and dermal exposure is explored with relation to the concept of bioavailability in the following sections.

3.1 Respiratory System

3.1.1 Structure and Function

The respiratory tract is a very complex system. Based on anatomic structure, particle deposition and clearance features, the respiratory tract is divided into three regions (Morrow *et al.* 1966):

- The nasopharyngeal region, including the nose, nasopharynx and larynx;
- The tracheobronchial region, containing trachea, bronchi, and bronchioles (diameter of 1 mm or less);
- The pulmonary region, comprising the respiratory bronchioles (diameter of about 0.06 mm), alveolar ducts, alveolar sacs, and alveoli surrounded by blood capillaries and lymphatic vessels.

In a new revised lung model proposed by the International Commission on Radiological Protection (ICRP) Task Group (Bair 1989, James *et al.* 1989), the thoracic lymph nodes are included in the pulmonary region, the so-called parenchymal-nodular region.

Absorption of xenobiotics may occur mainly in the alveolar epithelium because of the large surface area (140 to 150 m^2) (Gordon and Amdur 1991). The air-blood barrier (0.36 to 2.25 μm thick) consists of the aqueous layers on the surface of the alveoli, the epithelium cells (type I), the thin interstitial space containing fibroblasts, and the capillary endothelial cells (Phalen 1984).

The primary function of the respiratory system is gaseous exchange for uptake of oxygen and excretion of carbon dioxide. However, absorption, deposition, metabolism and clearance for xenobiotics also occurs in this organ. The structures and main functions for three regions are summarized in Table 3-1.

3.1.2 Rate-limiting Steps in the Gaseous Phase

Absorption of gases and vapors takes place in the entire respiratory tract but mainly in the alveoli. The transport processes can be divided into two steps (Figure 3-1):

- Transfer of the gas and vapor from the ambient environment into the alveoli, occurring in the *conducting airways;*
- Penetration of the *air-blood barrier* and dissolution in the blood.

Conducting Airways. Gases and vapors enter the respiratory tract following inhalation. This transport process is called dispersion. The velocity of convective airflow varies from region to region (Overton 1984, 1990). The maximum velocity usually occurs at the tube center in the trachea and at the inner wall of tube in the bronchi and bronchioles. The airflow, in the respiratory bronchioles, moves axially and slowly. Along the wall of the airways, there is a gas-liquid interface (aqueous layer). During the passage of gases and vapors, a small fraction will dissolve in this aqueous layer. This process is primarily dependent on the thickness of the aqueous layer, concentrations in the aqueous layer, and the gas-liquid partition coefficient of xenobiotics (Phalen 1984, Overton 1984, 1990).

Air-blood Barrier. Gases and vapors must pass through the air-blood barrier prior to reaching the systemic circulation. Gases and vapors exist as molecules, so that absorption occurs from gaseous diffusion following Fick's law (Guyton 1991).

Table 3-1 Main Physiological Functions of the Respiratory Tract

Region	Structure	Main Function
Nasopharyngeal		
Nose Nasopharynx Larynx	Mucous membrane Epithelium Glands	Warming and humidification of inhaled air Filtration of large inhaled particles Absorption of water-soluble compound Metabolism Olfaction
Tracheobronchial		
Trachea Bronchi Bronchioles	Mucous membrane Epithelium Cartilage Smooth muscle Glands	Conducting air and aerosols Deposition of medial particles Metabolism Fast particle clearance Uptake of gases and vapors
Pulmonary		
Respiratory bronchioles Alveolar ducts Alveolar sacs Alveoli	Single-layer epithelium with surfactant and capillaries	Gas exchange Deposition of small particles Absorption of gases, vapors and aerosols Slow or very slow particle clearance Synthesis and secretion of surfactant Metabolism

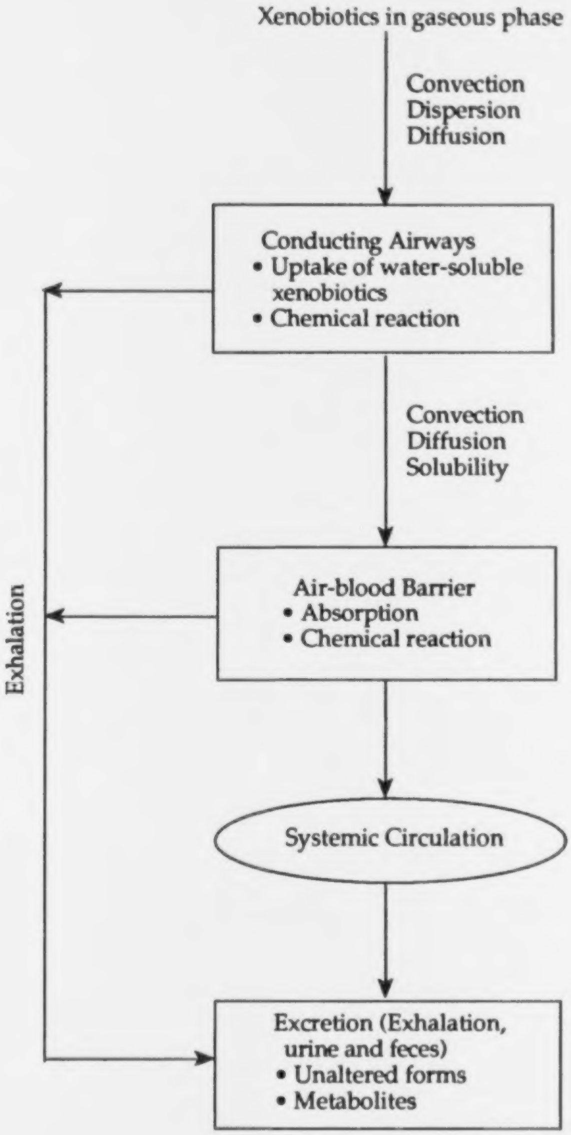
Xenobiotics in gaseous phase
Convection
Dispersion
Diffusion
Conducting Airways
• Uptake of water-soluble xenobiotics
• Chemical reaction
Convection
Diffusion
Solubility
Air-blood Barrier
• Absorption
• Chemical reaction
Systemic Circulation
Excretion (Exhalation, urine and feces)
• Unaltered forms
• Metabolites
Exhalation

Figure 3-1 Gaseous phase absorption of xenobiotics

The total resistance barrier to gaseous diffusion is the sum of resistances of the aqueous layer, epithelial layer, interstitial space and plasma fluid (Weibel 1970). With exposure to a gas or vapor for a sufficient time, tissues will effectively reach equilibrium. For diffusion in the liquid phase (aqueous layer, blood and other tissue fluids), the water solubility of a gas or vapor is a key determinant for absorption. At equilibrium, the solubility ratio of a xenobiotic between the blood and air is constant (Klaassen and Rozman 1991). This constant is termed as the "blood/air partition coefficient" ($P_{B/A}$):

$$P_{B/A} = \frac{C_B}{C_A} \tag{3-1}$$

where C_B and C_A are the concentrations of a xenobiotic in the blood and in the airspace, respectively. This partition coefficient should be inversely related to the Henry's law constant for the xenobiotic. Obviously, for a xenobiotic with a low $P_{B/A}$, the concentration of the xenobiotic that will dissolve in the blood will be low. In such a case, the blood will be rapidly saturated. Thus, an increase of rate of blood flow will enhance the rate of diffusion of the xenobiotic. This is a *perfusion limited step*. In contrast, a xenobiotic with a high $P_{B/A}$ is rapidly dissolved in the blood. The rate of xenobiotic diffusion is equal to the rate of xenobiotic removal by blood flow from the airspace, at equilibrium. Consequently, an increase of ventilation will accelerate the rate of absorption of a xenobiotic with high $P_{B/A}$, thereby providing a *ventilation limited step*.

3.1.3 Factors Influencing Respiratory Bioavailability for Gas and Vapor

In the case of a gas or vapor uptake by simple diffusion, the factors influencing diffusion will affect the respiratory bioavailability. Although the gas or vapor may diffuse in both directions across the barrier, the net diffusion is attributed to a variety of factors. These factors are pressure or concentration gradients, thickness of the air-blood barrier, surface area, water solubility, molecular weight, diffusion coefficient, blood/air partition coefficient, permeability of the membrane, ventilation, blood flow, chemical reactivity, and disease states. Of all the factors, the water solubility, blood/air partition coefficient, and chemical reactivity generally play the most important roles in determining the respiratory bioavailability for xenobiotics.

Water Solubility. There is a linear relationship between the solubility and the extent of deposition of a xenobiotic in the aqueous layer and tissue fluids (Overton and Miller 1988). The highly water-soluble and chemically reactive compounds tend to deposit to a high degree in the aqueous layer or tissue fluids of the upper respiratory tract unless the concentrations are extremely high (Cuddihy and McClellan 1989). Thus, such compounds are effectively removed to the aqueous layer from the conducting airway surface. These include compounds like sulfur dioxide, ruthenium, ammonia, iodine vapor (Pattle 1961, Speizer and Frank 1966, Black and Hounam 1968, Frank *et al.* 1969, Frank 1970, Webber and Harvey 1976, Kleinman, 1984). For lower solubility and reactive compounds (e.g. ozone), more than 50% of the inhaled gas or vapor may reach the pulmonary region (Miller *et al.* 1978, Cuddihy and McCellan 1989). Lipid-soluble and inert compounds (e.g. anesthetic) may primarily reach in the alveolar space, independent of their concentration in the air (Saidman 1983, USEPA 1989b). For the lipid-soluble compounds, the aqueous layer is a major barrier for absorption.

Pressure or Concentration Gradient. The pressure or concentration gradient refers to the difference between the partial pressure or concentration of a xenobiotic in the airspace of the alveoli and in the blood. When the pressure or concentration of the compound is greater on one side of the barrier than the other, the net diffusion will occur from the region of high pressure or concentration toward the region of the low pressure or concentration (Dinman 1991, Guyton 1991).

Thickness of the barrier. In terms of Fick's law, the rate of diffusion across the barrier is inversely proportional to the thickness of the barrier. The greater the thickness, the lower the rate of diffusion (Dinman 1991, Guyton 1991).

Surface Area. The rate of diffusion is a function of the alveolar surface area and the blood capillaries. Under resting condition, gas exchange is slightly affected with the loss of about two-third to three-fourth normal surface area of the alveoli, whereas the same loss of surface area will cause a serious problem to gas exchange during physical exercise (Guyton 1991).

Molecular Weight. The velocity of thermal motion of a dissolved gas is related to the square root of its molecular weight. The small molecules will be more permeable to the air-blood barrier (Bend *et al.* 1985, Guyton 1991).

Diffusion Coefficient. The diffusion coefficient (D) is correlated with the water solubility of a xenobiotic (S) and molecular weight (MW) (Phalen 1984):

$$D = \frac{S}{MW^{1/2}} \qquad (3\text{-}2)$$

Blood/air Partition Coefficient. See Section 3.1.2.

Permeability of Barrier. Permeability of the barrier is defined as the rate of diffusion of a xenobiotic through each unit area of the barrier for a unit concentration difference:

$$P = \frac{D}{A} \qquad (3\text{-}3)$$

where D is the diffusion coefficient and A is the total alveolar surface area (Guyton 1991).

Ventilation and Blood Flow. Ventilation and blood flow vary both at rest and during physical exercise. Normally, the ratio of ventilation (V_A) to blood flow (Q) remains constant although the ventilation or blood flow changes with time (Astrand 1983). This is termed as "ventilation-perfusion ratio", given by V_A/Q. However, at the beginning of physical exercise, the uptake of the gas and vapor is enhanced until the blood becomes saturated with the gas. When exercising at a constant work load or resting, the rate of absorption is constant.

Chemical Reactivity. Chemical reaction is of great concern for highly reactive gases and vapors. Using a mathematical model, Overton *et al.* (1987) predicted that 60% to 92% of ozone uptake, at different human-activity levels, occurred in the pulmonary tissues and only 2% to 3% reached the systemic circulation. The low systemic values are caused by chemical reactions between ozone and the lung tissues, which also produces pulmonary injury.

Disease State. Some pulmonary diseases cause edema and fibrosis in the interstitial space, which increases the thickness of the respiratory membrane, and emphysema, which decreases the surface area of the alveoli. As a result, these disease states reduce the rate of diffusion of the gas and vapor into the bloodstream (Bend *et al.* 1985, Guyton 1991).

3.1.4 Rate-limiting Steps in Particulate Phase

Evaluation of bioavailability for aerosols involves determining a series of processes: deposition, clearance and retention (Figure 3-2).

Once inhaled, aerosols may directly deposit on the surface of various regions of the airways. However, not all the airborne aerosols may be respired. Currently, the inhalable particulate matter (IPM) often refers to the size of aerosols less than 10-15 μm mass median aerodynamic diameter

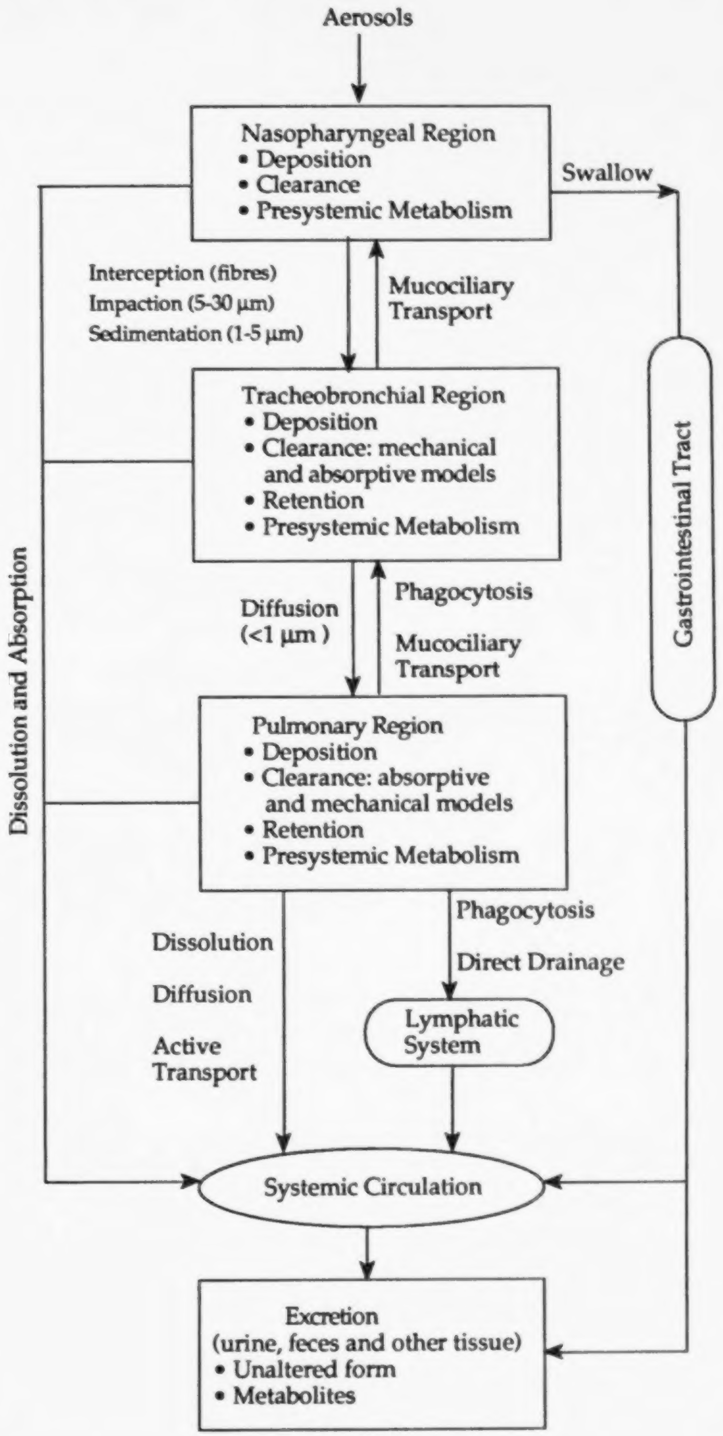
Aerosols
Nasopharyngeal Region
• Deposition
• Clearance
• Presystemic Metabolism
Swallow
Interception (fibres)
Impaction (5-30 μm)
Sedimentation (1-5 μm)
Mucociliary Transport
Tracheobronchial Region
• Deposition
• Clearance: mechanical and absorptive models
• Retention
• Presystemic Metabolism
Gastrointestinal Tract
Dissolution and Absorption
Diffusion (<1 μm)
Phagocytosis
Mucociliary Transport
Pulmonary Region
• Deposition
• Clearance: absorptive and mechanical models
• Retention
• Presystemic Metabolism
Dissolution
Diffusion
Active Transport
Phagocytosis
Direct Drainage
Lymphatic System
Systemic Circulation
Excretion
(urine, feces and other tissue)
• Unaltered form
• Metabolites

Figure 3-2 Deposition, retention and clearance of aerosols

(MMAD). This measure reflects the reality that sampling methods used to characterize particle size distribution depend upon the aerodynamic properties rather than absolute size of particles. This type of particulate matter, described as IPM_{10} or IPM_{15}, has the properties to reach the tracheobronchial and pulmonary regions (Lioy 1990b, USEPA 1990). The inhalable fraction, that is, intake efficiency or inhalability, is defined as the portion of the total mass of airborne aerosols that actually cross the nose and mouth. Vincent and Armbruster (1981) reported that 82% of aerosols less than 10 µm MMAD could enter the human respiratory tract, whereas the inhalable fraction for aerosols between 30 to 80 µm MMAD was only 50%. Paustenbach *et al.* (1991) reported a range of 6% to 30% of the inhalable fraction for chromium particulates less than 10 µm MMAD. USEPA data that were reviewed by Paustenbach *et al.* (1986, 1987) indicated that the inhalable fraction for aerosols less than 10 µm MMAD was only 30%, and that for total suspended particulates (TSP) was no more than 50%.

After being deposited, aerosols may be cleared from the respiratory system by mechanical and absorptive processes. The mechanical clearance is the removal of the deposited materials to pulmonary lymph nodes or to the oropharynx in which the xenobiotics are swallowed or expectorated. The absorptive process refers to the transfer of the dissolved inhaled materials from the respiratory tract or lymph nodes to the systemic circulation. The uncleared aerosols will be retained within the respiratory tract, i.e. retention, according to the relationship:

Retention = Deposition - Clearance.

A. Deposition

Deposition is defined as the process whereby the mass of the inhaled xenobiotic is deposited in various regions of the respiratory tract during inhalation exposure. The principal mechanisms of deposition include interception, impaction, sedimentation, and diffusion (Raabe 1979 and 1982) (Note that, in this section, the aerosol size parameters are aerodynamic diameters, MMAD, for aerosols ≥ 0.5 µm, and diffusion diameters for those < 0.5 µm that reflect the diffusion properties of aerosols):

- *Interception* at bifurcations of bronchial trees occurs only for long fibrous particulates (e.g. asbestos). In this case, the length and shape of particles are more important determinants of deposition than settling velocity.

- *Inertial impaction* means that particulates (ranging from 5 to 30 μm) continue to travel along their original direction because of their inertia despite changes in the airstream direction. Those larger particles can be impacted on the surface of the large airways.
- *Sedimentation* is the process whereby the small particles (1 to 5 μm) settle in the small bronchi, bronchioles and the alveolar space by gravitational force.
- *Diffusion* means that aerosols less than 1 μm (particularly <0.5 μm) will reach the surface of the small airways and alveoli primarily by diffusion.

Deposition is highly variable with the aerosol size distribution and breathing patterns (nasal inhalation and oral inhalation). In principle, inhaled aerosols between 5 to 30 μm, in humans, may be trapped in or pass thorugh the nasalpharyngeal region. Inhaled aerosols of 1 to 5 μm may be deposited in the tracheobronchial region. Aerosols less than 1 μm tend to reach the alveolar surface. Schlesinger (1989) analyzed a great variety of studies from the scientific literature. The results of total and regional deposition fractions for humans are described as:

- *Total deposition* for aerosols >0.5 μm is greater via nasal breathing than via oral breathing, whereas there is little difference in the total deposition for aerosols between 0.02 to 0.5 μm. The results indicated the maximum total deposition fraction is approximately 100% for aerosols of 8-10 μm; and about 20% for those of 0.3 to 0.5 μm. For aerosol sizes ranging within 0.5 to 8 μm, the total deposition increases with increasing aerosol size. For aerosol sizes less than 0.2 μm, the total deposition is inversely proportional to aerosol size.
- *Nasopharyngeal deposition* (the upper respiratory tract) is still greater by nasal inhalation than by oral inhalation. For instance, the fraction for 2 to 10 μm of aerosols is 40% to 90% via nasal inhalation, and only 2 to 35% via oral inhalation. The predominant aerosol size deposited in this region ranges from 1 to 10 μm, with most larger than 5 μm. Within this range, the deposition fraction increases with increasing aerosol size.
- *Tracheobronchial deposition* has been found to be relatively constant, ranging from 20% to 40% for aerosol sizes between 2 to 10

μm. As the aerosol size decreases to less than 1 μm, the deposition fraction markedly declines.

- *Pulmonary deposition* via oral breathing is proportional to the aerosol size larger than 0.5 μm. The deposition fraction for aerosols between 2 to 4 μm ranges from 30% to 60%, whereas aerosols between 0.1 to 1 μm have a deposition fraction between 15% to 20%. The deposition fraction via nasal breathing is relatively constant (20% to 22%) for aerosols of 0.7 to 3 μm. Conversely, when the aerosol size is larger than 3 μm, the deposition fraction reduces with an increase of aerosol size. Clearly, nasal breathing gives rise to lower deposition fractions in the pulmonary region of the respiratory tract for the larger size aerosols than oral breathing.

When data on the regional deposition fraction and particle size distribution are not known, ICRP (1968) assumes that 25% of total inhaled aerosols are exhaled, 50% are deposited in the nasalpharyngeal and tracheobronchial regions, and 25% reach the pulmonary region. About 60% of inhaled aerosols appear to be swallowed into the gastrointestinal tract (Kimbrough *et al.* 1984, Paustenbach *et al.* 1986, and Sheehan *et al.* 1991).

B. Clearance

Clearance of aerosols can be divided into the two processes:

- *Non-absorption processes* (mechanical translocation). These processes usually occur for soluble aerosols and relatively insoluble aerosols that very slowly dissolve in the fluid of the respiratory tract.

- *Dissolution-absorption processes* Water soluble aerosols undergo these processes. The solubility of the deposited aerosols is a dominant factor for absorption.

Water Insoluble Aerosols

Generally, insoluble aerosols tend to be retained within the respiratory tract to cause the pulmonary injury and diseases, by chemical reactions and metabolism. These deposited aerosols are removed by various processes, depending on the deposition regions.

In the nasopharyngeal region, the major clearance processes are mucociliary transport, sneezing, wiping and blowing. Aerosols deposited in the oral passage may be swallowed into the gastrointestinal tract. The clearance half-time (time for 50% removal) by sneezing, wiping and blowing is estimated as less than 30 minutes, and mucociliary transport is about 12 hours (Fry and Black 1973, Morrow 1977, Schlesinger 1989, USEPA 1989c).

In the tracheobronchial region, insoluble aerosols are cleared by mucociliary transport and coughing. Clearance rates vary considerably with the depth of the airways. The average clearance half-time is 48 hours (ranging from 25 minutes to 5 months) (Bailey *et al*. 1985). About 1% of the deposited aerosols in this region are likely to be retained long-term and ultimately penetrate into the deep tissues (epithelium, gland, and connective tissue) (Brain and Mosier 1977, Watson and Brain 1979, Gore and Patrick 1982, Schlesinger 1989).

Insoluble aerosols from the pulmonary region undergo alveolar-macrophage associated particle clearance and lymphatic system clearance.

Alveolar-macrophage Associated Particle Clearance. After ingestion by alveolar macrophages, aerosols may be moved upward to the oropharynx, followed by swallowing into the gastrointestinal tract. Clearance rates in this step is aerosol-size-independent but depends on the rate of phagocytosis. The clearance half-time is estimated as weeks (slow particle clearance) (Schlesinger 1988, 1989).

Lymphatic System Clearance. Either the ingested or free insoluble aerosols may be cleared from lymphatic drainage via direct penetration or phagocytosis within a few hours or days (Schlesinger 1988, 1989). Clearance rates for free aerosols in this step are size-dependent (Raabe *et al*. 1978, Stradling *et al*. 1978). These aerosols may be immobilized and accumulated in the lymph nodes which serve as reservoirs for the systemic circulation, with the clearance half-time of months (Schlesinger 1988, 1989).

Water Soluble Aerosols

In addition to the mechanical clearance, the dominant clearance of the soluble aerosols in each region takes place via dissolution-absorption process.

Dissolution-absorption Clearance. Water soluble constituents eluted from the matrix of particles may be initially dissolved within the alveolar aqueous layer and directly pass the air-blood barrier, by simple diffusion, into the systemic circulation. Alternatively, the aerosols may be engulfed by alveolar macrophages and dissolved within the macrophages. Then, the dissolved constituents are released out of the macrophages and become available for absorption. In specific cases (e.g. paraquat), the absorption occurs by an active transport process. The clearance rates in this step are very slow, taking months or years (long-term clearance or very slow clearance) (Cuddihy and Yeh 1988).

Typically, the absorption rate is dissolution-dependent and aerosol-size-dependent. Highly water soluble aerosols appear to be rapidly passed through aqueous paths or pores in the air-blood barrier following deposition, particularly in the nasopharyngeal region where there is an abundant vascular network. The rate of absorption is inversely related to the molecular size (Mercer 1967).

Conversely, lipophilic compounds can readily penetrate the air-blood barrier. The rate of absorption varies linearly depending on the octanol/water partition coefficient. The absorption rate of weakly acidic or basic compounds is determined by their degree of ionization in the aerosol fluid or alveolar aqueous layer. The non-ionized form will behave more lipophilic.

In some cases, small fractions of dissolved constituents from aerosols may remain in the specific local regions for a long period, by binding the cellular molecules of the pulmonary tissue (Mewhinney and Griffith 1982, Cuddihy 1984). This process can reduce the absorption of the deposited substances, but may promote cytotoxicity to the pulmonary tissue.

C. Retention

Following repeated inhalation exposure to aerosols for a sufficient time, the rate of deposition and clearance will reach a balance. At equilibrium, the rate of retention of aerosols in the respiratory tract remains constant. The total mass of the retained aerosols is defined as the "equilibrium lung burden", which may indicate the relative concentrations in exposure air. On the other hand, if the rate of clearance is less than that of deposition, the lung burden increases, which is termed as "lung overloading". Because of the interactions, the retention pattern can be used to predict the equivalent concentration of exposure aerosols, that is, the concentrations of aerosols that would produce equivalent deposition of inhaled aerosols per unit mass of lung (Snipes 1989).

3.1.5 Factors Influencing Respiratory Bioavailability for Aerosols

Respiratory bioavailability for aerosols is dependent on both deposition and clearance. These processes are affected by a number of variables. In general, the variables can be categorized into five groups: (1) environmental characteristics; (2) physicochemical characteristics; (3) respiratory tract characteristics; (4) breathing pattern characteristics; and (5) general characteristics.

Physicochemical factors involve particle size, shape, density, surface area, hygroscopicity, electrical charge and conductivity, dissolution rate, solubility and ionization. As mentioned previously, the structure of the various parts of the respiratory tract is related to determine the site of deposition and the rate of clearance. Also, breathing patterns, such as respiratory rate, volume of inhaled air, air velocity and breath-holding, dramatically influence the deposition and clearance of aerosols. Moreover, general factors (age, gender, and disease states) have to be considered for respiratory bioavailability studies. Of all these factors, the properties of aerosols are the most critical for determining the deposition and clearance behaviour. Environmental factors include temperature, humidity, composition of air, and wind velocity. Environmental factors will not be discussed further.

Particle Size. As discussed in Section 3.1.4, particle size is a key determinant for deposition and clearance processes.

Shape of Particle. The shape of particles will influence deposition pattern and phagocytic efficiencies (Mercer 1967, Raabe 1971, Phalen 1984, Cuddihy and Yeh 1988). Particles with an irregular shape have a large surface and low density. As a result, they may deliver much greater amount of xenobiotics into the alveoli.

Density. If the geometric diameters of two aerosols are the same, the settling velocities are directly proportional to density (Mercer 1967, Raade 1971, Phalen 1984, Cuddihy and Yeh 1988).

Surface Area. As aerosols become smaller, there is a marked increase in surface area as well as in physicochemical activities (Mercer 1967, Raade 1971, Phalen 1984, and Cuddihy and Yeh 1988). The surface area of aerosols will affect the ability of aerosols to adsorb gases and the rate of dissolution of particles in the respiratory tract (Phalen 1984). An increase in surface area of particles appears to enhance the toxicity because there is a larger exposed surface for reaction with cells or tissues (Hatch and Gross 1964).

Hygroscopicity. Some water-soluble aerosols will grow in moist air until they reach equilibrium, e.g. sea salt, tobacco smokes and sulfuric acid droplets. Typically, the smaller particles grow more rapidly than the larger ones. At equilibrium, the size of hygroscopic particles can be utilized to estimate inhalation dose and deposition pattern (Ferron 1977, Phalen 1984).

Dissolution Rate. In principle, the dissolution rate is proportional to the surface area of aerosols (Cuddihy and Yeh 1988). The smaller particles may dissolve and be cleared faster than the larger ones. Also, dissolution makes constituents of the particle available for metabolism within the respiratory tract.

Solubility and Extent of Ionization. As discussed in Section 3.1.4, these two factors will influence the penetration of the air-blood barrier for aerosols.

Anatomy. The diameter of the airway influences the displacement required by the aerosols (Snipes 1989, Schlesinger 1985, 1990). The airflow velocity is attributed to the cross section of the airways.

Breathing Pattern. During exercise, an increase of respiratory rate and inhaled volume or breath-holding may accelerate the settling velocity and rate of deposition (Palmes *et al.* 1973). Using oral breathing, the larger aerosols may be delivered into the deeper respiratory tract.

Metabolism. The respiratory system also contains a group of biotransformation enzymes. Detoxication and toxication of aerosols may occur before a xenobiotic reaches the systemic circulation, which is called "respiratory presystemic metabolism" (Benford and Bridges 1986). However, this effect is very small, and generally insignificant for assessing the bioavailability for most xenobiotics.

Age. Mucociliary transport rate appears to be slow with age (Clarke and Pavia 1980).

Disease States. Pulmonary diseases may decrease the rate of aerosol clearance by various mechanisms (Brain 1980).

3.2 Gastrointestinal System

3.2.1 Structure and Function

The gastrointestinal (GI) tract comprises the esophagus, stomach, small intestine (the duodenum, jejunum and ileum) and colon. In general, the typical structure of the wall of the gastrointestinal tract consists of the four main components (Smith 1986, Kutchai 1988, Selen 1991):

- *Mucosa* that contains an epithelial cell layer with goblet cells and endocrine cells, the lamina propria in which there are lymph nodules and capillaries, and the muscularis mucosae;
- *Submucosa* that is constituted by loose connective tissue, with glands and the larger blood vessels of the gastrointestinal wall;
- *Muscularis externa* that consists of two layers of smooth-muscle cells;
- *Serosa* that is outermost layer from the interior of the gastrointestinal tract.

The small intestine, the main absorptive site, comprises the typical structure along with special surface features: crypt, villi and brush border with the unstirred water layer, microvilli (apical membrane) and glycocalyx. Microvilli that increase the mucosal surface area contain various digestive enzymes (disaccharidases and peptidases). The major barrier to the transport of xenobiotics is the mucosal epithelial cell layer, intercellular space, the tight junction which is located at the juxtaluminal region and forms a continuous barrier, and capillary endothelial cells.

The physiological function of the gastrointestinal tract varies greatly from site to site along its length. The main functions of various regions are summarized in Table 3-2.

3.2.2 Rate-limiting Steps

After entering the gut lumen and, if possible, dissolving in the gut fluid, a xenobiotic may cross the gut/blood barrier (mucosal barrier), via transcellular or intercellular route, by one of the two major mechanisms of gastrointestinal absorption: passive (simple diffusion and filtration) and active transport. Absorption of macromolecules may be involved in

Table 3-2 Main Physiological Functions of the Gastrointestinal Tract

Region	Structure	Main Function
Esophagus	*Typical structure with squamous epithelium	Delivery of food
Stomach	*Typical structure with more circular muscle layer (pH 1 - 3.5)	Mixing Emulsion formation Protein digestion Absorption of certain lipid-soluble compounds
Small Intestine	*Typical structure with certain surface feature (crypt, villi, microvilli)	Absorption
Duodenum	Presence of Brunner's gland in the submucosa (pH 5 -6)	Influx of biliary and pancreatic secretions Iron and calcium absorption
Jejunum	(pH 6 - 7)	Hydrolysis of disaccharides Absorption of neutrients, water, eletrolytes and certain chemicals
Ileum	(pH 7 -8)	Absorption of bile salt, sodium, vitamin B_{12} and certain chemicals
Colon	Absence of well-defined villi	Absorption of water and sodium Secretion of potassium

*Typical structure: mucosa, submucosa, muscularis externa, and serosa.

phagocytosis, pinocytosis and persorption (Houston and Wood 1980, Csáky 1984, Aungst and Shen 1986, Abdou 1989, Klaassen and Rozman 1991, Dawson 1991). A xenobiotic that crosses the gut/blood barrier appears in the portal blood, passes through the liver, and then enters the systemic circulation. A xenobiotic that is not absorbed is eliminated from feces. The absorptive processes are depicted in Figure 3-3.

Unstirred Water Layer. This mucous covering (300 to 400 nm of thickness) is the initial diffusion barrier for xenobiotics (Houston and Wood 1980, Csáky 1984, Thomson and Dietschy 1984, Hänninen 1986). Water-soluble xenobiotics pass through this layer by simple diffusion following Fick's law. For hydrophobic xenobiotics, this layer will represent the rate-limiting barrier.

Transcellular Route. The absorptive cell layer (approximate 1 μm of thickness) is constituted by a series of membranes: the apical membrane, the basolateral membrane and the basement membrane of the epithelial cell, and the wall membrane of the lymphatic or blood capillary (Esposito 1984a). The hydrophobic substances readily permeate this layer by diffusion (Capraro 1984).

A variety of nutrients and endogenous compounds, for instance, sugar, amino acid, bile salt, minerals, water-soluble vitamin and certain xenobiotics (lead and aluminum), are known to be absorbed from the small intestine by the active transport process (Stein 1984, Foulkes 1984, Esposito 1984b). Furthermore, macromolecules may be absorbed via pinocytosis.

Intercellular Route. There is an intercellular route between the two epithelial cells, including the tight junction, the gap conjunction, the pores, the intercellular space, the basement membrane of the epithelial cell, and the wall membrane of the lymphatic or blood capillary (Esposito 1984a). Fluid absorption has been considered to occur through aqueous pores (0.3 to 0.88 nm) by diffusion, filtration, osmosis and active transport. Small molecules may penetrate the pores of the membrane or the tight junction with this water flux (Turnheim 1984). Large molecules, e.g. starch granules and diatomaceous earth particles, may enter the intercellular space from a gap at the tip of the villi between two cells which are sloughed off. This process is termed persorption (Csáky 1984, Aungst and Shen 1986).

First Pass Effect. This effect has been described as presystemic xenobiotic metabolism. Some xenobiotics will be metabolized at the applied sites (the airway wall, the gut wall and the skin) or during passage through the liver

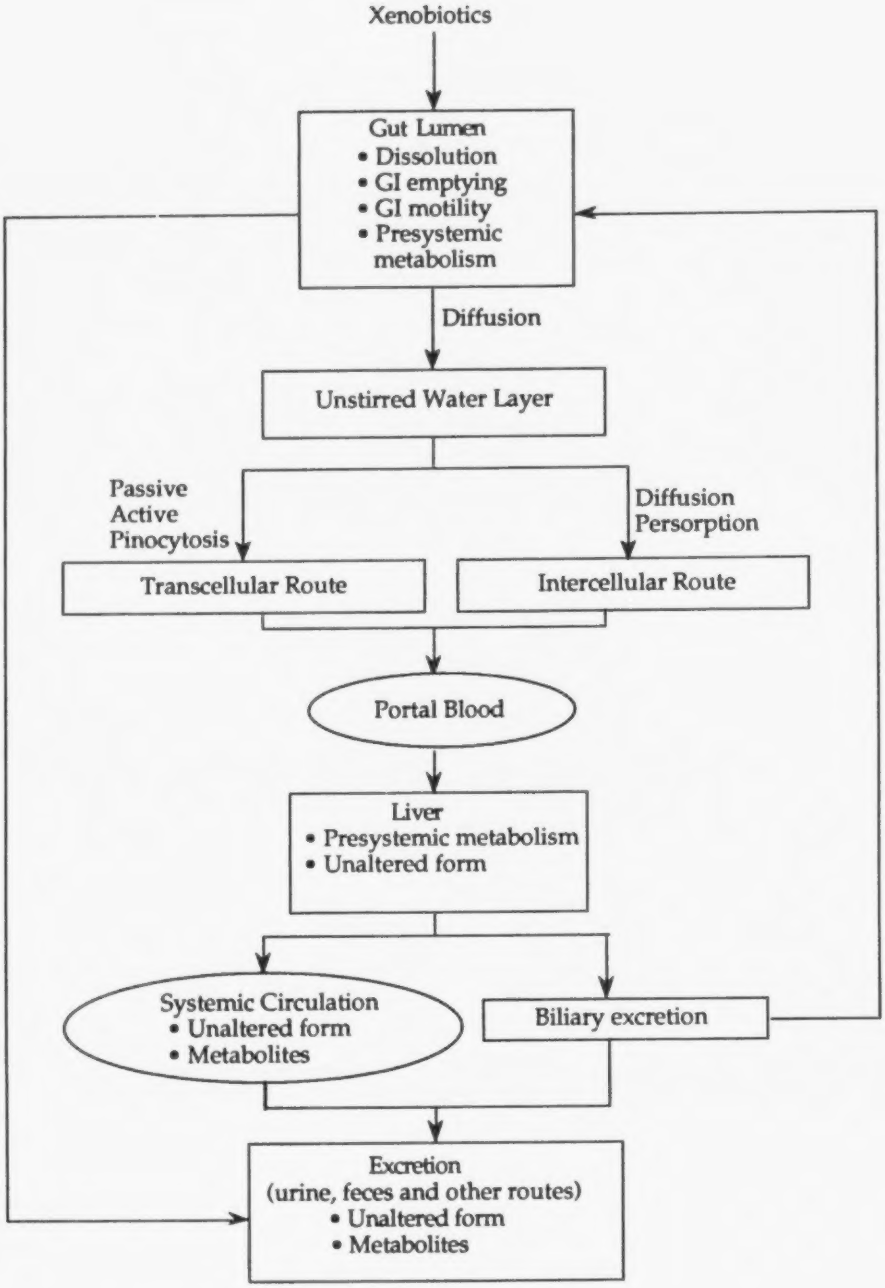
Xenobiotics
Gut Lumen
• Dissolution
• GI emptying
• GI motility
• Presystemic metabolism
Diffusion
Unstirred Water Layer
Passive
Active
Pinocytosis
Diffusion
Persorption
Transcellular Route
Intercellular Route
Portal Blood
Liver
• Presystemic metabolism
• Unaltered form
Systemic Circulation
• Unaltered form
• Metabolites
Biliary excretion
Excretion
(urine, feces and other routes)
• Unaltered form
• Metabolites

Figure 3-3 Absorptive steps via ingestion

from the portal vein prior to the systemic circulation (Abdou 1989, Labaune 1989, Dinman 1991, Selen 1991). The result of the first-pass effect is a reduction of the total mass of xenobiotics that can reach the systemic circulation. Usually, the hepatic first-pass effect will dramatically influence the oral bioavailability for most xenobiotics in humans because the liver is the primary metabolic organ. As compared to the liver, in most cases, the first-pass effect in the applied sites is less quantitatively significant.

3.2.3 Factors Influencing Oral Bioavailability

The bioavailability of a xenobiotic via the ingestion route varies greatly, depending on a variety of factors. These factors include physicochemical (water solubility, octanol/water partition coefficient, dissolution rate, molecular size, dosing forms, vehicles and complexation), physiological (gastrointestinal emptying and motility, blood flow, first-pass effect, and enterohepatic circulation), and general factors (age, gender and diseases). Of all the factors, the dissolution rate, gastrointestinal emptying and motility, blood flow, first-pass effect are the key determinants for the oral bioavailability.

Dissolution Rate. The dissolution rate of solid xenobiotics in the gastrointestinal tract has been found to be one of the rate-limiting factors in gastrointestinal absorption (Houston and Wood 1980, Smolen and Ball 1984, Welling 1984, 1988, Abdou 1989, Labaune 1989, Dinman 1991, Selen 1991). The fundamental equation for dissolution rate (DR) is described by the Noyes-Whitney law:

$$DR = K\,(C_s - C) = \frac{DS}{h}\,(C_s - C) \qquad (3\text{-}4)$$

where K is a dissolution rate constant or partition coefficient, C_s is the solubility of the xenobiotic in solvent, C is the concentration of dissolved substance in the bulk medium, D is the diffusion coefficient of the solute molecules in the medium, h is the thickness of the diffusion layer, and S is the solid surface area.

The dissolution rate may be altered by the diffusion coefficient, surface area of particles, thickness of film, dissolution constant and solubility. However, a key determinant of dissolution is the solubility of xenobiotics. Because a low water-solubility solid particulate or nonpolar compound dissolves poorly in the aqueous gastrointestinal fluids, it may not be effectively absorbed in spite of the ease of penetration of cell membranes.

The solubility can be altered by the entire dissolution medium in the gastrointestinal fluid or the diffusion layer surrounding a particle. Thus, pH range in the gastrointestinal fluid may affect the dissolution rate. Also, the dissolution rate is a function of the surface area of a particle.

Furthermore, the dosing form and vehicle are important considerations in the dissolution process. For example, salts of weakly acidic or basic compounds are dissolved and absorbed faster than are the free acids or bases.

Gastrointestinal pH. Gastrointestinal pH may influence the absorption of weak acids or bases (Labaune 1989, Dinman 1991, Klaassen and Rozman 1991, Selen 1991). Based on the Henderson-Hasselbalch equations, the bioavailability for an organic acid or base is related to the degree of its nonionized form that relies upon the dissociation constant, as:

For weak acids

$$\log \frac{[\text{nonionized}]}{[\text{ionized}]} = pK_a - pH \qquad (3\text{-}5)$$

For weak bases

$$\log \frac{[\text{ionized}]}{[\text{nonionized}]} = pK_a - pH \qquad (3\text{-}6)$$

In the acidic environment (i.e. stomach), the nonionized form (more lipid-soluble) for a weak acid is predominant, and is more readily absorbed. In the alkaline environment (i.e. the small intestine), a weak base is more easily absorbed. Conversely the rate of absorption will be slower for a weak acid. However, because of the huge surface area of the small intestine, the overall capacity of absorption for weak acids is still greater. Generally, the presence of food or certain compounds could alter the pH range of the gastrointestinal tract.

Gastrointestinal Emptying and Motility. Gastrointestinal emptying and motility have been recognized as controlling steps in gastrointestinal absorption (Johnson 1992). To some extent, the degree of absorption is proportional to the xenobiotic residence time. The food types may delay, reduce or increase the xenobiotic absorption because of the changes of the gastrointestinal emptying and motility. For instance, liquid meals have shorter residence times than solid meals. In the fasting state, the emptying and motility rates may increase, resulting in an increased absorption of xenobiotics.

Blood Flow. Blood flow has been considered to be a rate-limiting step for the absorption of highly lipophilic xenobiotics (Labaune 1989, Dinman 1991, Selen 1991). After crossing the intestinal epithelium, the xenobiotic is rapidly removed by blood flow which maintains a concentration gradient (i.e. sink condition). This is a *perfusion rate-limited process*. As a result, bioavailability is proportional to blood flow. In contrast, the absorption of hydrophilic xenobiotics is usually independent of blood flow and is controlled by diffusion, that is, a *diffusion rate limited process*.

Interaction and Complexation. A xenobiotic may interact with the intestinal mucin, bile acid, inorganic salts and other chemicals in the gastrointestinal tract and form a complex which is incapable of penetrating the mucosal barrier (Gregus and Klaassen 1986). Thus, the complex may inhibit the absorption of xenobiotics. For instance, cadmium decreases zinc and copper absorption. Phosphate and calcium may inhibit absorption of aluminum. The complexation may also increase the extent of absorption. Lipophilic xenobiotics or xenobiotics with oily vehicles in the small intestine may form micelles with bile salts. Those mixed lipid-bile salt micelles are more easily absorbed. Lead absorption may be enhanced by oral administration of a chelating agent, like EDTA.

First Pass Effect. see Section 3.2.2.

Enterohepatic Circulation. This is a process whereby xenobiotics and their metabolites entering the intestine with biliary excretion may be reabsorbed into the portal blood and returned to the liver (Gregus and Klaassen 1986). This effect may influence both the bioavailability and disposition of the xenobiotic by prolonging the residence time of the xenobiotic in the body.

Age. In humans, infant and young children may absorb some xenobiotics faster than adults (e.g. lead absorption via ingestion). On the other hand, the elderly appear to absorb some xenobiotics more slowly (Greenblatt *et al*. 1982, Eller 1992).

Disease State. Diseases or gastrointestinal surgery could markedly alter the bioavailability of xenobiotics (Smolen and Ball 1984).

3.3 Skin

3.3.1 Structure and Function

The human skin comprises two distinct tissues: dermis and epidermis, with appendages (hair follicles, sweat and sebaceous glands) (Barry 1983, Klaassen and Rozman 1991):

- *Dermis* (3 to 5 mm thick, the deepest layer) is a matrix of connective tissue containing blood vessels, nerves and lymphatics, in which any xenobiotic can be removed into the systemic circulation.
- *Epidermis* (50 to 100 μm thick) consists of proliferating cells and serves as the primary barrier to the absorption of substances through the skin. The outermost layer of the epidermis known as the *stratum corneum* (10 to 15 μm thick) is composed of 10 to 15 layers of cornified cells with cross-linked keratin filaments, i.e. a nonpolar lipid matrix surrounding a large number of thin parallel protein plates (the so-called brick-and-mortar model). This lipid matrix contains much more neutral lipids and low phospholipid content, as compared to the living epithelial tissue (Lampe *et al.* 1983).

The skin has many varied functions. The most important one is the protective function acting as the barrier to xenobiotics, microorganisms, radiation, heat and other foreign agents. The stratum corneum is considered as the crucial rate-limiting external barrier in dermal absorption of xenobiotics. Furthermore, the skin serves as an important xenobiotic metabolizing organ.

3.3.2 Rate-limiting Steps

Percutaneous absorption is defined as the xenobiotic transport through the epidermis into the dermis to reach the systemic circulation *in vivo*. For *in vitro* study (particularly by using the non-viable skin), xenobiotics penetrate the skin barrier by diffusion, accumulate on the other side of skin, and are not removed by the systemic circulation. This process is described as skin permeation.

The mechanism of percutaneous absorption for all xenobiotics is likely to be passive diffusion and none is involved in the active transport process

(Klaassen and Rozman 1991). The steady-state transport of lipophilic chemicals through biological membranes is a solubility-diffusion process (Aguiar *et al*. 1969, Barry 1983, Smith 1990, Potts and Guy 1992). The xenobiotics initially dissolve into the surface layer of the stratum corneum, then diffuse into the stratum corneum, the rest of epidermis and dermis, respectively. Thereafter, they partition into the body fluid at the stratum corneum-epidemis interface, epidermis, and dermis, respectively, and then ultimately enter the systemic circulation (see Figure 3-4).

Based on experimental data, there are two possible transport routes for percutaneous absorption (Barry 1983, Idson and Behl 1987, Flynn 1990, Behl *et al*. 1990, Illel *et al*. 1991):

Transappendageal route. The appendages provide an aqueous pore pathway. For very hydrophilic xenobiotics, this route may play an important role in dermal absorption.

Transepidermal route. This includes intercellular and transcellular pathways. The intercellular (lipid partitioning) pathway for penetration is through the lipid of the stratum corneum. The transcellular pathway for permeation is through the aqueous tissue of the skin.

For non-polar xenobiotics, there is a linear relationship between skin permeability and the physicochemical properties of the xenobiotics in accordance with solution-diffusion theory and experimental data (Blank *et al*. 1967, Blank and Scheuplein 1969, Ackermann *et al*. 1987, Hatanaka *et al*. 1990, Potts and Guy 1992). The rate of permeability of these compounds is mainly related to their solubility and octanol/water partition coefficients, and is inversely proportional to the molecular weight (Marzulli *et al*. 1965, Lieb and Stein 1971, Potts and Guy 1992). The diffusion coefficients within the lipid phase are significantly lower than within the porous phase (Barry 1983). Thus, this kind of partition-limiting behavior may play an important role in the skin permeation although the space of lipid phase contributes only 10% to 30% of the total stratum corneum volume (Grayson and Elias 1982). The existence of the lipid pathway is useful for developing simple lipid membranes as a model skin barrier (Houk and Guy 1988).

For polar xenobiotics, the characteristic of the permeability can be better described by pore theory. Based on studies of the hydrophilic drugs

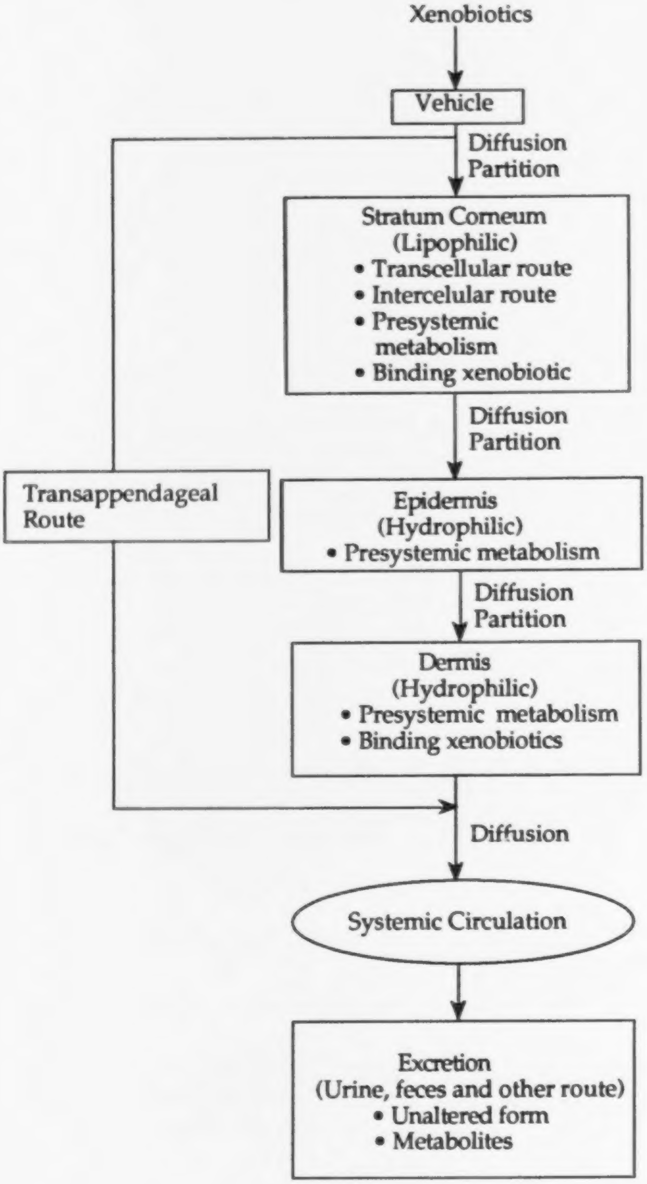
Xenobiotics
Vehicle
Diffusion
Partition
Stratum Corneum
(Lipophilic)
• Transcellular route
• Intercelular route
• Presystemic metabolism
• Binding xenobiotic
Diffusion
Partition
Transappendageal Route
Epidermis
(Hydrophilic)
• Presystemic metabolism
Diffusion
Partition
Dermis
(Hydrophilic)
• Presystemic metabolism
• Binding xenobiotics
Diffusion
Systemic Circulation
Excretion
(Urine, feces and other route)
• Unaltered form
• Metabolites

Figure 3-4 Absorptive steps via dermal route

(Ackermann *et al.* 1987, Hatanaka *et al.* 1990), the permeability coefficients of these compounds through hairless mouse skin or artificial porous membrane depend on their molecular size, rather than their octanol/water partition coefficients.

Consequently, the stratum corneum provides a two phase lipid-protein heterogeneous membrane with parallel lipophilic and hydrophilic pathways. Once a xenobiotic crosses the stratum corneum, it partitions into the underlying layers of epidermis, dermis and systemic circulation, which are more hydrophilic than the stratum corneum. These underlying layers can be a barrier for lipophilic compounds.

3.3.3 Factors Influencing Dermal Bioavailability

The factors influencing rate of skin absorption include the physicochemical factors of the xenobiotic (such as structure, molecular weight, pKa, ionization, stability, volatility, solubility, partition coefficients and diffusivity) and physiological factors (such as skin hydration, metabolism, age, skin condition and site of application). These factors can be examined by reference to published values, theoretical calculations or experimental measurements.

The most important parameters for a xenobiotic are its water solubility, permeability coefficient, octanol/water partition coefficient and diffusivity.

Water solubility. Water solubility will directly limit the rate of permeability for lipophilic xenobiotics (Scheuplein 1965, Aguiar *et al.* 1969, Ravis 1990, Flynn 1974, 1990).

Octanol/water Partition Coefficient (k_{ow}). The k_{ow} is used to predict the relative rate of skin absorption from partitioning experiments between two immiscible two liquid phases, typically octanol/water or mineral oil/water (Ravis 1990, Flynn 1974, 1990). The k_{ow} and water solubility measurement can provide a good indicator of solubility in the membrane, but cannot predict diffusivity.

Diffusivity. Based on Fick's law of diffusion, the permeability rate of an xenobiotic is proportional to diffusivity (Ravis 1990, Flynn 1974, 1990). Diffusivity is a kinetic property that depends on the state of substance (e.g. gas, liquid, or solid), temperature, viscosity, polarizability, electronic effects, hydrophobicity and molecular size. Nevertheless, the difference between diffusion coefficients is relatively small compared to difference in the k_{ow}.

Permeability Coefficient. Dermal permeability is experimentally measurable. The permeability coefficients of some organic compounds from water or vapor for humans are available in the literature (Dutkiewicz and Piotrowski 1961, Hanke *et al.* 1961, Piotrowski 1967, 1971, Blank *et al.* 1967, Dutkiewicz and Tyras 1967, 1968, Scheuplein and Blank 1971, 1973, Roberts *et al.* 1977, Albery and Hadgraft 1979, Baranowska-Dutkiewicz 1982, Southwell *et al.* 1984, Blank and McAuliffe 1985, Wieczeorek 1985, Bronaugh *et al.* 1986, Hadgraft and Ridout 1987). These values, however, can only be gathered under steady-state conditions. Thus, the permeability is commonly applied for evaluating human exposure to vapors or to waterborne contaminants during swimming and bathing. Although the permeability may be considered as a constant over time, it is highly variable according to the nature of chemicals (molecular size and shape, hydrophobicity and solubility), concentration of chemicals applied to skin, and thickness of skin barrier.

Ionization. The surface of human skin is observed to be weakly acidic (pH 4.2 to 5.6), while the lower layers have a more neutral pH of 7.4. Penetration of acidic and basic organic xenobiotics is influenced by the pH of the skin or a carrier vehicle (Smith 1990). Generally, a weak acid placed on the relatively acidic skin will occur mainly in the unionized form that will more readily penetrate the skin. If the pH of the skin is changed by other media, the properties of the skin may also be altered, resulting in changes of the dermal absorption. Extremes of pH can damage the structure of the skin and dramatically alter dermal bioavailability.

Hydration. Skin hydration (wet skin) may cause the tissue to soften, swell or wrinkle. This state can increase the permeability rate of the skin (Barry 1983).

Metabolism. Some xenobiotics (e.g. some carcinogenic PAHs) are sensitive to enzymatic metabolism within the skin (Scheuplein and Bronaugh 1983, Barry 1983, Ravis 1990). They may be metabolized. The resulting reactive metabolites may covalently bind with DNA or protein in the skin tissue to exert local toxicity or carcinogenicity. Thus, the dermal bioavailability (access to systemic circulation) will be reduced.

Age. Infants or young children demonstrate much greater bioavailability than adults because of their under-developed skin barrier (Fisher 1989). The skin absorption of certain compounds may be lower in the elderly (Roskos *et al*. 1989, Hall *et al*. 1992).

Skin Condition. A diseased or damaged skin usually increases permeability and the resultant rate of skin absorption (Scheuplein and Bronaugh 1983, Barry 1983, Ravis 1990).

Site of Application. The skin absorption in humans or animals varies with the site of chemical application, which is termed regional variation (Wester and Maibach 1989a, Gorsline *et al*. 1992). Permeability in the genital area or scrotum and around the head and face is higher than that in the limbs. The foot or palm areas exhibit much lower in permeability.

Physical Form. The xenobiotic may be exposed to the skin with a vehicle which may be solid, aqueous, aqueous paste, suspension, volatile, solvent or gas. The properties of the vehicle can be one of the major factors influencing dermal bioavailability (Meyer 1991, Hugest *et al*. 1992).

Dermal Decontamination. In most cases, a soap-and-water wash will remove contaminants from the skin. However, there is also evidence that dermal decontamination for certain xenobiotics may enhance the skin absorption (Wester and Maibach 1989b).

3.4. Summary

Humans may be exposed to environmental contaminants via inhalation, ingestion and dermal contact. The mechanisms of absorption for each exposure route were summarized from the literature and discussed. The absorptive barriers and rate-limiting determinants are unique for each exposure route and are summarized in Table 3-3.

The exposure state, physicochemical or physiological properties of xenobiotics and biological membranes affect the bioavailability. Various factors influencing bioavailability in humans are summarized in Table 3-4.

Table 3-3 The Barriers and Rate-limiting Processes for Absorption of Xenobiotics through Three Exposure Routes

Exposure route	Barrier	Rate-limiting determinant
Inhalation	Air/blood barrier: (1) aqueous layer; (2) epithelial cell; (3) interstitial space (4) capillary endothelial cell	Gas/vapor Water solubility Blood/air partition coefficient Chemical reactivity Aerosol Water solubility of a xenobiotic in the aerosol Dissolution rate in the respiratory tract Particle size distribution
Ingestion	Gut/blood barrier: (1) mucosal epithelial cell; (2) tight conjunction; (3) intercellular space; (4) capillary endothelial cell	Dissolution rate Blood flow Gut motility First-pass effect
Dermal	Stratum corneum	Lipid solubility Permeability Octanol/water partition coefficient (k_{OW}) Diffusivity

Table 3-4 Factors Influencing Bioavailability in Humans

Exposure factor	Physicochemical factor	Physiological factor
Concentration	Solubility	Age
Duration	Partition coefficient	Race
Dosing vehicle	pKa	Gender
Dosing form	Dissociation rate	pH of site application
Frequency of exposure	Molecular size	Surface area
Species difference	Molecular weight	Permeability of barrier
Environmental condition (temperature, humidity, and wind velocity)	Reactivity	Specialized transport
	Polarity or solubility	Interaction with mucous
	Diffusion coefficient	Complexing components
	Volatility	Local blood flow
	Ionization	Surface activity
	Partial pressure	Bile interaction
	Vapor pressure	Food effects
	Properties of particle (shape, density, surface area, electric charge and hygrosocopicity)	Gastric emptying
		Hydration of skin
		First-pass effect
		Motility of GI
		Absorption sites
		Plasma protein levels
		Metabolism
		Recirculation
		Clearance
		Ventilation
		Breathing pattern
		Disease states

4. Experimental Methods for Bioavailability

Both *in vivo* and *in vitro* methods have been used in the bioavailability studies in humans and animals.

Because *in vivo* methodology is inherently more representative, this approach is greatly preferred for assessing the bioavailability. However, the direct administration of xenobiotics into the human body may pose a hazard for humans. In a few cases, such studies have been conducted with young healthy male adult volunteers (Capel *et al.* 1972), but ethics approval for non-medical trials are very unlikely to be approved in future. More realistically, laboratory animals are utilized for these studies. Because indirect methods are most commonly used, there will be substantial uncertainties involved with interpreting the results. Additionally, biological variables, individual organism variability, animal care ethics and high cost must be resolved.

There are a variety of *in vivo* methods for investigating the bioavailability via the three main exposure routes. Indirect methods (e.g. measurement of xenobiotics in biological samples) are conducted in living animal models, whereas direct methods (e.g. mass-balance technique) require the animals to be sacrificed to confirm the extent of absorption.

Attempts have been made to develop *in vitro* methodology for bioavailability studies. As compared to *in vivo* methods, *in vitro* methods are less expensive, less time-consuming, and involve fewer ethical issues. Good agreement of *in vivo* and *in vitro* for some xenobiotics has been observed. Currently, most *in vitro* methods are not directly applicable for the quantitative bioavailability. Nevertheless, *in vitro* methods are widely used for the determination of some important physicochemical factors, such as solubility, water/air or lipid/water partition coefficient, dissolution rate and particle size distribution.

4.1 General *In Vivo* Methods

4.1.1 Measurement of Blood Levels

Measurement of blood levels of xenobiotics is often employed to quantitatively evaluate the bioavailability for most xenobiotics through all three main exposure routes (Barr 1972, Wester and Noonan 1978, Nugent and Wood 1980, Hwang and Danti 1983, Wester *et al.* 1983a, McDougal *et al.* 1985, Aungst and Shen 1986, Ogiso *et al.* 1987, Abdou 1989, Hall and Shah 1990). The procedure is described as:

- Subjects are prepared (test animals).
- A single dose or multiple doses of a tested xenobiotic, often labeled with radioactive substances, are administered via (1) extravascular (ev) routes, including inhalation, ingestion and dermal, and (2) intravenous (iv) route. The intravenous dose is used as a reference dose because it is directly introduced into the systemic circulation and may be assumed to have a bioavailability factor equal to one.
- Serial blood samples are collected at desired intervals until absorption and distribution are completed.
- Blood levels of the xenobiotic are measured by using appropriate analytical techniques. Chromatographic techniques and liquid scintillation spectrometry are commonly employed for the qualitative and quantitative analysis of xenobiotics in biological samples.
- Blood levels are plotted verse time to determine the area under the plasma concentration-time curve (AUC), in units of mass x time/volume (Abdous 1989):

$$AUC = \int_{T0}^{T\infty} C_i \, dt \qquad (4\text{-}1)$$

where C_i is the plasma concentration of the xenobiotic.

- The bioavailability factor (BF) is calculated from:

For a single exposure

$$BF = \frac{AUC_{ev}/D_{ev}}{AUC_{iv}/D_{iv}} \ (\times\ 100) \qquad (4\text{-}2)$$

in which D_{ev} refers to extravascular dose, D_{iv} is intravenous dose, assuming that the excretion rate constants of both administration routes are consistent.

For multiple exposures

$$BF = \frac{(AUC_{n(ev)}/D_{ev})\tau_{ev}}{(AUC_{n(iv)}/D_{iv})\tau_{iv}} (\times 100) \tag{4-3}$$

in which τ is the dosing time.

For relative bioavailability (Labaune 1989)

$$BF_R = \frac{AUC_2}{AUC_1} (\times 100) \tag{4-4}$$

in which AUC_1 is the blood concentration for a standard material, and AUC_2 is the blood concentration for a test material.

Strengths:

- This approach is the most sensitive and accurate way for the assessment of both the extent and rate bioavailability.

Limitations:

- This method is inconvenient for small animal studies because of the small total volume of blood in these animals.
- This method can be technically demanding.

4.1.2 Measurement of the Cumulative Urinary Excretion

Because the blood concentration and the urine excretion concentration are proportional, the measurement of the cumulative concentration of the xenobiotics in urine is used for roughly estimating the bioavailability. Typically, the urinary data represents the lower bound of bioavailability factor for most xenobiotics (Abdou 1989). The procedure is similar to the blood levels:

- Subjects are prepared (test animals).
- A tested xenobiotic is administered via extravascular routes and intravenous route.
- Serial urine samples are collected at desired intervals until excretion is completed.

- Urinary levels of the xenobiotic are measured by using appropriate analytical techniques.
- The bioavailability factor is calculated from:

$$BF = \frac{A_e^{\infty}(ev)/Dev}{A_e^{\infty}(iv)/Div}\ (\times 100) \qquad (4\text{-}5)$$

in which A_e^{∞} is the total mass of a xenobiotic excreted in urine from dosing time to infinity.

For relative bioavailability (Labaune 1989)

$$BF_R = \frac{A_e^{\infty}2}{A_e^{\infty}1}\ (\times 100) \qquad (4\text{-}6)$$

in which $A_e^{\infty}1$ is the total mass of a xenobiotic in urine for a standard material, and $A_e^{\infty}2$ is the total mass of a xenobiotic in urine for a test material.

Strengths:

- This method is simple and convenient in animal studies by taking urine samples.
- This method is inexpensive and rapid.

Limitations:

- This method may not be reliable. The approach usually underestimates the bioavailability factor in most cases because of the presence of a small fraction of the parent compound in urine and other variables, such as the urinary pH being different from blood pH, amount of free form in plasma, urinary flow rate, collection methods and sensitivity of analytical methods.
- This method is valid only when at least 20% of the total dose is eliminated in urine following intravenous administration.

4.1.3 Mass-balance Technique

Recently, the mass-balance technique has been extensively employed for bioavailability studies in animals (Pegg *et al.* 1979, Schumann *et al.* 1980, Shah *et al.* 1981, 1987, Frantz and Watanabe 1983, Shah and Guthrie 1983, Reifenrath *et al.* 1984a, 1984b, Bucks *et al.* 1988, Hall and Shah 1990, Schlesinger 1990, Banks and Birnbaum 1991, Hughes *et al.* 1992, Tsai *et al.* 1992). This is based on conservation of xenobiotic mass knowing the administered dose. Because all the xenobiotics presented in the systemic circulation will be distributed to various body fluids, organs and tissues, the total mass of a xenobiotic in these sites other than the applied sites represents the internal dose. Thus, the internal dose is the total recovery minus the mass associated with the site of application. The procedure is:

- Subjects are prepared (test animals).
- A xenobiotic is administered by inhalation, ingestion or dermal contact.
- Blood and urine samples are collected at desired intervals.
- Animals are sacrificed at selected times.
- The xenobiotic is analyzed in the body fluids, tissues and organs to determine the mass recovery.
- The ratio of internal dose (D_{in}) to external dose (D_{ex}) is calculated to determine the bioavailability factor, as:

$$BF = \frac{D_{in}}{D_{ex}}(\times 100) \qquad (4\text{-}7)$$

where D_{in} includes the mass of xenobiotics in the body fluids, tissues and organs.

Strengths:

- This method is accurate for assessing the bioavailability factor without the need of a correction (i.e. intravenous administration) for considering the unknown excretion and quantifying the metabolites of xenobiotics.

Limitation:

- This method is complex.
- This method is expensive and time-consuming.
- This method provides limited information on kinetic factors.

4.1.4 Measurement of Toxic Response

The occurrence of toxic effects, either local or systemic, may indicate the absorption of xenobiotics. Thus, in some cases, a specific toxic response can be used to estimate the extent and rate of bioavailability (Gaines 1960, 1969, Gaines and Linder 1986). Nevertheless, this approach is less accurate and quantitative for assessing the bioavailability.

4.2 Specific Methods for the Inhalation Exposure Route

Bioavailability within the respiratory tract is a very complex process. For evaluating gas and vapor uptake, the water solubility of gas and vapor and the total intake are often examined. For aerosols, the assessment of bioavailability is associated with a series of chain events: deposition-clearance-retention. Bioavailability may be estimated by calculation of the difference between the deposition fraction and the following fractions: retention fraction, and mechanical clearance fraction, with a correction for the amount of xenobiotic cleared to the gastrointestinal tract.

In principle, there are both *in vivo* and *in vitro* methods available for the study of respiratory bioavailability.

With *in vivo* methods, the exposure approaches include (1) *Whole Body Exposure*. Humans and animals are placed in a room or in a chamber (only for animals) supplied by the fresh air containing the test substance (Phalen *et al*. 1984, MacFarland 1987, Drew 1988, Dorato 1990, Snelling and Dodd 1990, Karg *et al*. 1992); (2) *Nose-only Exposure*. Animals are placed in stocks or whole-body tubes, and then the nose protrudes into the chamber (MacFarland 1987, Henry and Kouri 1987, Holländer 1988); (3) *Head-Only Exposure*. This is similar to nose-only exposure except that the animals are restrained in the chamber by a collar around the neck, so that the mouth, eyes and head are exposed to the test substance; and (4) *Intratracheal Instillation*. In the method, a blunt needle is inserted to 1 or 2 mm above the bifurcation of the airway and the test substances are directly introduced into the respiratory tract (Bend *et al*. 1985, Bevan and Ruggio 1991).

Recently, only one *in vitro* method, the isolated perfused lung, has been frequently used for bioavailability studies.

4.2.1 Determination of Deposition Fraction

After inhalation exposure, only the fraction of a xenobiotic deposited within the respiratory tract is available for further mechanical clearance and absorption. Consequently, the deposition fraction is related to the applied dose.

4.2.1.1 Gaseous Phase

Typically, characterization of an inert insoluble gas or vapor (such as CO), including mixing and distribution, can be determined by lung function tests (Ultman 1988). For toxic and reactive gases or vapors, the deposited pattern in various regions are derived from mathematical models, rather than from experiments (Miller *et al.* 1985, 1988, Overton 1984, 1990, Overton *et al.* 1987, Cuddihy and McClellan 1989).

Deposition of gases and vapors in the wall of airways accounts for the reduction of concentrations in the pulmonary region. Thus, the applied dose is less than the administered dose. The applied dose rate (J_{app}) is the product of the alveolar ventilation rate ($\dot{V}_A$) and the difference between the concentrations in the inhaled air (C_{in}) and in the exhaled air (C_{out}) (Baskin and Falco 1989), expressed as:

$$J_{app} = \dot{V}_A (C_{in} - C_{out}) \qquad (4\text{-}8)$$

In most experiments relevant to gas/vapor uptake, determination of the total intake (i.e. deposition coefficient) is common. In some cases, total intake is used for estimating the bioavailability factor. The procedure is described as:

- Subjects are prepared (test animals).
- A tested xenobiotic is administered via whole body exposure, nose-only exposure and head-only exposure.
- The xenobiotic concentrations in the inhaled air and in the exhaled air are measured.
- Deposition fraction (total intake) is calculated, as expressed by

$$I = \frac{C_{in} - C_{out}}{C_{in}} (x\ 100) \qquad (4\text{-}9)$$

Strengths:

- This approach is simple and convenient.

Limitations:

- The bioavailability factor is overestimated for most xenobiotics because the total intake reflects the fraction of the total inhaled xenobiotic deposited within the respiratory tract, rather than those across the air-blood barrier.
- The bioavailability factor is applicable only for the relatively low water-solubility xenobiotics because of the absorption of gases and vapors being water-solubility-dependent. The highly water-soluble xenobiotics tend to deposit on the aqueous layer of the upper airways. The lipophilic xenobiotics primarily reach the alveoli where there is a large surface available for absorption and the compounds readily penetrate the air-blood barrier. Therefore, the deposition fraction for highly lipophilic xenobiotics will also represent the bioavailability factors.
- The bioavailability factor is variable with breath time, blood/air partition coefficient, and blood flow rate.

Moreover, the determination of some physicochemical factors, such as blood/air partition coefficient and water solubility of the xenobiotics, is required for interpreting the bioavailability study.

4.2.1.2 Particulate Phase

Total deposition fraction can be determined by comparing the difference of concentrations between the administered test aerosols in the inhaled air and in the exhaled air. Regional deposition fraction may be estimated by the measurement of aerosol concentrations in the different volume fraction at the exhaled air, by making assumptions about mixing and dead space (Schlesinger 1985, 1988, 1989). Alternatively, deposition fraction may be estimated from inhalable fraction and particle size distribution.

A variety of analytical techniques are applied for assessing the aerosol size distribution, such as optical or electron microscopy, cascade impactor, nuclei counters, charge spectrometer, light extinction device, diffusion battery, surface area measurement device and elutriators (Macias and Husar 1976, Phalen 1984, Holländer 1988, Cai *et al*. 1992, Cheng *et al*. 1992, Hunt *et al*. 1992).

4.2.2 Mechanical Clearance Fraction and Retention Fraction

The majority of aerosols deposited within the respiratory tract will be cleared by mechanical processes. Clearance studies generally focus on examining the rate for removal of inhaled xenobiotics from the whole lungs (the portion of tracheobronchial region and the entire pulmonary region) and from individual airways, alveolar macrophage function, and accumulation in the lymph nodes. Measurement of the long-term clearance rate to lymph nodes is rarely available in human studies. Such data may be derived from animal studies. The common methods for assessing mechanical clearance are:

- *Mucociliary Transport Velocity*. Mucociliary transport rate, which refers to the time required for aerosols to move between two defined areas, can be identified by the monitoring of markers (e.g. colloid albumin, India ink droplet, color solution and dye) on the epithelium, or measuring the movement of radioactive aerosols within the airways (Schlesinger 1990). This technique is simple but invasive.

- *Whole Lung Clearance*. There are two methods for measurement of whole lung clearance (Lippmann 1981, Schlesinger 1990). First, the clearance rate can be determined by using a scintillation detection technique. The second technique involves the use of magnetopneumography. The aerosols which produce magnetization can be detected by magnetopneumography (Cohen 1973, Cohen *et al*. 1979, Halpern *et al*. 1981).

- *Fecal Analysis*. Following inhalation of radioactive aerosols, feces samples collected at selected times are analyzed by liquid scintillation detector (Kenoyer *et al*. 1981, Mannix *et al*. 1983). This measurement reflects the xenobiotic clearance by the mucociliary transport into the gastrointestinal tract.

- *Macrophage Function*. Classic approaches for studying the pulmonary macrophage function consist of ultrastructure, biochemistry, *in vitro* culture, and immunologic bioassays. The *in vivo* procedure for assessing phagocytosis is a comparison of the difference between free gold and cell associated gold washout pattern after administration of labeled colloidal gold (Beck*et al.* 1981). Recently, magnetic particles and sensitive magnetometers are applied for studying the pulmonary macrophage motility *in vivo* (Brain 1992).

4.2.3 Dissolution Rate

Dissolution testing is used to study the behavior of aerosols when the dissolution is a rate determinant for absorption.

- *In vivo methods*. The radioactive particles are injected into the muscle of the experimental animals, where the test substances are dissolved in the biological fluids. The clearance profiles can be obtained by analyzing the retention of the test substances at the injected site. The clearance parameters from the intramuscular injection were found to correspond to data from the lung (Morrow *et al.* 1968, 1979, Thomas *et al.* 1973). The intramuscular injection procedure provides information about the dissolution-absorption clearance.

- *In vitro methods*. The constituents of the aerosols may be eluted by using ultrafiltration with buffered and unbuffered biological methods, and other techniques (Bevan and Worrell 1985, Bevan and Yonda 1985, Törnquist *et al.* 1988, Bevan and Ruggio 1991).

4.2.4 Absorption Fraction

- *In vivo methods*. See Section 4.1.1, 4.1.2 and 4.1.3.

- *In vitro methods*. See Section 4.2.5.

4.2.5 *In Vitro* Method: Isolated Perfused Lung

The isolated perfused lung preparation is well suited to the investigation of xenobiotic bioavailability and metabolism in the lung (Mehendale *et al.* 1981, Niemeier 1984, Rohades 1984, Rao and Mehendale 1987, Niven and Byron 1988, Roth and Bassett 1989). The procedure involves:

- An isolated lung is prepared by removal of the lung from an experimental animal.

- A perfusion system is prepared, including a negative pressure ventilation-perfusion system (Niemeier and Binghham 1972), positive pressure ventilation-perfusion system (O'Neil and Tierney 1974), and a split lung perfusion system (Tucker and Shertzer, 1980).
- The isolated lung is placed in the perfusion apparatus where it is suspended by the pulmonary arterial cannula.
- The medium is perfused into the pulmonary arterial cannula at a constant flow and pressure.
- Serial samples are collected.
- Concentrations in the collected samples are measured by appropriate analytical techniques.

Strengths:

- The pulmonary structure and function integrity is maintained.
- The role of the lung, compared to the whole animal or lung slice preparation, is evaluated.
- Mass balance of xenobiotics may be determined by measuring the mass recovery in the perfusate, the tissue and the exhaled air via the trachea.
- Exposure routes are similar to those under physiological conditions. Test xenobiotics may be administered by inhalation, pulmonary artery, or trachea.
- A variety of compositions of perfused medium may be used.
- Experimental parameters, such as volume of perfusion medium, concentration of test substances, perfusion pressure, ventilation rate, and blood flow, may be easily controlled.

Limitation:

- The perfused lung is only viable for a short time.
- It is technically demanding to set up and conduct the experiment.

4.3 Specific Methods for the Ingestion Exposure Route

In principle, there are three broad categories for determining the gastrointestinal bioavailability.

- *In vivo* methods, involving unanesthetized animals, provide direct assessment of the bioavailability factors of xenobiotic via ingestion. In addition to the basic methods mentioned in Section 4.1, the measurement of the cumulative fecal excretion data is frequently used. The other methods consist of the measurement of liver ratio of a xenobiotic, and chronic isolated loop model.

- *In situ* methods, involving anesthetized animals, need to take the intestine outside animals but keep the blood supply intact. The methods provide the experimental conditions to mimic the *in vivo* conditions. This approach may be used to study the mechanism of transport, site of absorption and gastrointestinal metabolism. The techniques contain perfused loop, closed loop and mesenteric collection.

- *In vitro* methods, involving isolated gastrointestinal tissue, dissolution and partition coefficient tests, are referred to mode membranes that more closely reflect the functionality of biological membranes. The isolated gut wall methods are usually referred to model membranes which more closely reflect the functionality of biological membranes, and are used to study the transport characteristic of the substance whose absorption is a passive process. These techniques comprise everted intestinal sac (it has been turned inside out), isolated perfused intestinal segment, and biological and artificial membrane models. Dissolution and partition coefficient tests are also applicable for determining physicochemical factors relevant to the bioavailability

The latter two categories, *in situ* and *in vitro* methods, only provide give us an insight into the intestinal membrane-level screening of the xenobiotic absorption potential.

4.3.1 Measurement of Fecal Excretion

Using cumulative monitoring of a xenobiotic in feces is very common for for assessing the gastrointestinal bioavailability (Abdous 1989, Cutler 1986, Labaune 1989, Notari 1987). The value from this method reflects the disappearance of a xenobiotic from the gastrointestinal tract. The procedure includes:

- Subjects are prepared (test animals).
- A tested xenobiotic is administered via the oral route (feed or gavage).
- Serial feces samples are collected at desired intervals.
- The fraction of the total unaltered compound in feces (f_f) that is not absorbed is monitored.
- The fraction of the xenobiotic entering the portal blood (F') is calculated by the following equation:

$$F' = 1 - f_f \tag{4-10}$$

F' is the fraction of a xenobiotic that appears in the portal blood. The estimation of fraction of presystemic metabolism in the liver (f_h) for most xenobiotics is needed for an assessment of systemic availability. Estimation of f_h is relatively simple in animals but more difficult in humans. The equation for f_h is given by:

$$f_h = \frac{CL}{Q} \tag{4-11}$$

in which CL is the hepatic clearance and Q represents the hepatic blood flow. Thus, the bioavailability factor is

$$F = F' - f_h = 1 - f_f - f_h \tag{4-12}$$

For instance, when a xenobiotic completely crosses the gut wall into the portal blood (that is, $f_f=0$ and $F'=1$) and the fraction of the hepatic presystemic metabolism is 0.7, the bioavailability factor will be 0.3.

Furthermore, the hepatic presystemic metabolism tends to be easily saturable. Consequently, the bioavailability factor is dependent not only on the concentration of a xenobiotic in the portal blood but also on the saturability of the hepatic metabolism. Under this circumstance, the extent of absorption in the systemic circulation is nonlinearly proportional to an increase of the administered dose.

Strengths:

- This approach is relatively simple and convenient.

Limitations:

- The bioavailability factor will be overestimated without a correction of the fraction of the hepatic presystemic metabolism for those xenobiotics that are readily metabolized in the liver. This will be substantial in many cases.

4.3.2 Measurement of Liver Ratio

Oral bioavailability may be estimated by comparing the ratio of the levels of a test form to those of a standard form for the same xenobiotic in the liver following oral administration in animals (McConnell *et al.* 1984, Umbreit *et al.* 1986, Shu *et al.* 1988). The approach is based on the premise that the level of the xenobiotic in the liver may represent the systemic level of the xenobiotic in the body and that the bioavailability of the standard material is assumed to be 100%. Some studies indicated that this approach was applicable for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Shu *et al.* 1988). First, the liver is a major organ to uptake and retain TCDD, so that the liver levels are likely to reflect the systemic levels of TCDD. Second, the levels of TCDD in the liver slowly decrease after orally dosing, so that significant quantities of TCDD may be measured.

4.3.3 Thiry-Vella Loop or Chronic Isolated Loop

This technique is performed with unanesthetized large animals (e.g. dogs) as well as small animals (Ings 1984, Csáky 1984, Poelma and Tukker 1987, Lu *et al.* 1992). A segment of the intestine, with its blood supply and lymphatic connection intact, is isolated and attached to the abdominal wall. Subsequent to abdominal suture and surgical recovery, the animal is restored to a normal diet. The test substance is introduced into the loop and samples from the loop content are collected at selected times. A correction is made for water flux by examination of changes in concentration of a nonabsorptive marker.

4.3.4 Dissolution Testing

Dissolution testing, for determination of physicochemical factors, is extensively employed to predict the oral bioavailability of solid xenobiotics. Most techniques of estimating water solubility are derived from bioavailability studies for drugs, such as USP rotating basket, rotating paddle, column flow-through apparatus, sartorius apparatus (*in vivo* simulative method), BioPredictor (in-vivo predictive methods), and equilibrium geochemical speciation model MINTEQA 2 (Smolen and Ball 1984, Ruby *et al.* 1992, Davis *et al.* 1992).

During the past decades, scientists attempted to establish *in vitro-in vivo* correlation of the compounds by using *in vitro* bioavailability studies. In some selected cases, dissolution tests may predict *in vivo* behavior. Most of those have been unsuccessful. However, the *in vivo* environment is more complex than the *in vitro* test environment. *In vitro* dissolution tests cannot reliably predict differences among substances for poor *in vivo* bioavailability, superbioavailability and changes in bioavailability by physiological factors.

4.3.5 Methods Based on the pH Partition Theory

Based on pH partition theory, the relative oral bioavailability of a xenobiotic can be predicted by comparing its partition coefficients in two immiscible solvent. A number of methods are reviewed by Abdou (1989). These methods include inverted Y-shaped tube, circular cell, aqueous-octanol models, finite-difference methods, and two phase model with dissolution layer. Inverted Y-shaped tube and circular cell methods are restricted to the study of xenobiotics in solution.

4.4 Specific Methods for Dermal Exposure Route

Specific *in vivo* methods for dermal bioavailability are the investigation of the surface disappearance of a xenobiotic at the applied area of the skin. Besides *in vivo* methods, *in vitro* methodology is a powerful tool for evaluating the dermal bioavailability. Because the stratum corneum is nonliving and the rate limiting membrane, *in vitro* approaches are likely to accurately measure the skin bioavailability and reflect *in vivo* skin absorption profiles, with the ability to control the experimental conditions.

The waterborne contaminants or vapors in contact with the skin can directly penetrate the skin barrier into the systemic circulation. Because there are difficulties with determining the contact rate for waterborne contaminants or vapors, it is very difficult to apply a bioavailability factor. Rather, the extent of absorption can be determined by dermal permeability. In most cases, the permeability coefficients are derived from *in vitro* tests. For some hydrophilic compounds, good agreement between *in vivo* and *in vitro* dermal absorption has been found. This correlation is poorly understood for lipophilic and extremely hydrophilic compounds (USEPA 1992b).

4.4.1 Surface Disappearance

Because a xenobiotic directly penetrates through the skin into the systemic circulation, the dermal bioavailability can be estimated from the examination of the surface disappearance (Hall and Shah 1990). The bioavailability factor is the difference between the applied dose and the fraction of a xenobiotic that disappears from the applied site. Several appropriate techniques for measuring the surface disappearance have been reported in the literature. In principle, these procedures investigate the loss and retention of a xenobiotic within the skin in humans and animals and provide the dermal absorption profiles.

- *Tape Stripping*. The stratum corneum is stripped off by successively sticking it to cellophane tape (20 to 30 times) following a short application periods (30 minutes). The tape strippings are assayed by using appropriate methods (Baker and Kligman 1967).

- *External Counting Technique*. A radiation detector is placed over the application site and the radiation from a labeled xenobiotic is quantified (Marty *et al*. 1989, Hall and Shah 1990). This technique is easy to use, noninvasive, objective, precise and inexpensive.

- *Histological Method*. A biopsy of the site of application is taken at a desirable time and the location of the compounds is determined under microscopy (Harris *et al*. 1974, Hall and Shah 1990, Srikrishna *et al*. 1992). This technique is strictly qualitative.

4.4.2 *In Vitro* Method: Biological Membrane Models

Biological membranes involve living or nonliving skin in humans or animals (Behl *et al*. 1990, Bronaugh 1990). Human skin is preferred for most skin permeation studies. Human skin is obtained from autopsies (fresh cadaver skin) or surgical specimens (face, breast, and abdominal). Depending on the need of the study, whole skin, stripping skin, epidermis and dermis could be chosen. However, human skin is not readily obtained for experimental purpose. A number of animal skins, including hairless mouse, rat and guinea pig, Swiss mouse, Athymic nude mouse, furry rat, nude rat, cat, dog, rhesus monkey and rabbit, have been extensively used for skin permeation studies.

Some researchers have been exploring new approaches for skin permeation studies (Itoh 1990a, 1990b, Rigg and Barry 1990, Harada *et al*. 1992).

Currently, shed snake skin, which is a nonliving pure stratum corneum with no follicles, has been investigated as a model membrane. Compared to rat or mouse skin through which the permeability coefficients are usually 100-1000 fold greater than that through human skin, the data from shed snake skin are only several times different from human skin. Overall, shed snake membrane may be a good model membrane system for the study of human skin permeability in terms of the similarities to human skin, low cost, and ease of storage and handling.

In model membrane studies, the thickness of the membranes can play a critical role in influencing the skin permeability. In general, there are several ways to prepare the membranes.

- *Full-thickness Skin Resection*. This method consists of the epidermis and the full-thickness dermis (even subcutaneous tissue) and is suitable for thin skin animals (e.g. mouse and rabbit) (Bronaugh and Stewart 1984).

- *Split Resection*. Human and some animal skins (guinea pig, pig, and monkey) are almost 1 mm or more than 1 mm thick and need to be split into thinner sections for experiments. This section of skin contains the viable epidermis and a variable quantity of the capillary dermis, with at least approximately 200 µm thick including the microcirculation (Barry 1983).

- *Isolated Perfused Porcine Skin Flap (IPPSF)*. This is a living *in vitro* method which uses the intact skin of a pig with a viable and defined arterial and venous supply (Riviere and Monteiro-Riviere 1991). The preparation, a single-pedicle axial pattern tube, may be perfused under ambient environmental conditions. The tested xenobiotic can be placed on the surface of IPPSF and assayed from the venous effluent over time.

- *Congenitally Athymic (Nude) Animal Models*. This skin model consists of a split thickness human or pig skin that is grafted to the subcutaneous surface of the epigastric skin of the nude mouse or rat (Klain and Black 1990). Following the application of a tested xenobiotic, animals are sacrificed and a variety of biological samples are analyzed.

Currently, there are two basic designs in the model membrane studies: the static cells and the flow-through cells (Frantz 1990, Gummer and Maibach

1991). The typical experiment consists of three compartments (diffusion cell): the donor, the barrier and the receptor. The two chamber systems can be divided into either the air/fluid phase chamber in which the surface of the epidermis exposes to the room air and the dermis is bathed in the aqueous medium, or the fluid/fluid phase chamber in which the aqueous medium is in contact with both sides of the skin preparation. The receptor fluid should be chosen to allow free diffusion of the xenobiotic into it. The normal saline and aqueous buffer solution are widely utilized. The test material is delivered to the membrane from the donor compartment, penetrates through the barrier and appears in the receptor compartment. The parameters are determined by sampling and analyzing the solute in either the donor or receptor phase as a function of time. The data can be expressed as either absorptive rate vs. time or cumulative absorption vs. time. In the aqueous/aqueous phase static diffusion cells, the parameters are evaluated by serial sampling of the receptor compartment, while the flow-through diffusion cells can provide automatic replenishment of the receptor liquid.

The xenobiotic samples are quantified by various analytical methods, such as radioactive detectors, chromatography, spectrophotometer, fluorescence and specific assays (Loden 1990).

The permeability, diffusion coefficient and partition coefficient can be determined from these experiments.

4.4.3 *In Vitro* Method: Artificial Membrane Models

Artificial membrane systems are generally divided into two categories: a liquid barrier and a solid barrier. In the liquid membranes which mimic the lipid nature of biomembranes, organic liquids (isopropyl myristate and tetradecane) are soaked on filter membranes (Albery *et al.* 1976, Albery and Hadgraft 1979, Guy and Fleming 1979, Hadgraft and Ridout 1987, 1988, Houk and Guy 1988, Ridout and Guy 1988, Tanaka *et al.* 1978). In contrast, solid membranes are involved in employing particular polymers or other solid materials as a barrier between two liquid phases.

Based on the mechanism of transport, two types of solid barriers are distinguished.

The first type is termed a dialysis membrane (heterogeneous barriers) in which the substances can penetrate through pores in the membrane without solution within the membrane. As a result, the rate of transport depends on the relative size of molecules and the tortuosity of the barrier pores (effectively diffusional path). Such a system may be representative of diffusion through damaged skin (dermis) (Houk and Guy 1988). This membrane system includes cellulose acetate (Gary-Bobo *et al.* 1969, DiPolo *et al.* 1970, Barry and El Eini 1976, Barry and Brace 1977), and membranes of biological origin, e.g. egg shell (Washitake *et al.* 1980) and collagen membrane (Nakano *et al.* 1976).

The second type is called the partitioning membranes (nonporous polymers) which act as a homogeneous barrier to free diffusion, involving the solution of the substance within the membrane. The rate of permeability is determined by the absolute water solubility, the relative affinity of the substance for membrane and the specific molecular structure (Garrett and Chemburkar 1968a). Thus, such a system is used as a lipoidal barrier of the stratum corneum. The most common membranes are Silastic® (polydimethyl siloxane) and polyurethane (Behl *et al.* 1984, 1985, 1986, Bottari *et al.* 1977, Flynn and Smith 1972, Flynn *et al.* 1979, Garrett and Chemburkar 1968a, 1968b, 1968c, Hunke and Matheson Jr., 1981, Kincl *et al.* 1968, Lovering *et al.* 1974a, 1974b, Most 1970).

The multimembrane system refers to the combination of two or more different membranes into a composite, such as cellulose acetate: silastic:cellulose acetate (Nacht and Yeung 1985) and silicone:poly (2-hydroxylethyl methacrylate) (Hatanaka *et al.* 1992).

The other less extensively studied membranes include Zeolites (Dyer *et al.* 1979), Diaflo-ultrafiltration membrane (O'Neill 1980) and Supor® (Behl *et al.* 1990).

Diffusion cell design, detection methods and data interpretation are similar to those for the biological membranes.

4.5 Summary

Either *in vivo* or *in vitro* methods are applicable for the study of bioavailability. The bioavailability factor measured for a xenobiotic may vary according to the different experimental methods used to measure it and the subjects involved. The use of *in vivo* methods in humans is unlikely because of the ethical considerations. Most experimental values are obtained from animal experiments. Hence, these values must be interpreted with caution during the use of such data for evaluating health risk assessment. The experimental methods for measuring bioavailability are summarized in Table 4-1.

Table 4-1 Choice of Experimental Methods for Study of Bioavailability

Method	Technique	Administered route	*Subject	Objective	Strength	Limitation
In vivo	Measurement of blood level	Inhalation Ingestion Dermal	Humans Animals	Bioavailability factor Accurate	Inconvenient Reliable	
In vivo	Measurement of urinary level	Inhalation Ingestion Dermal	Humans Animals	Bioavailability factor (lower bound)	Simple Convenient Inexpensive Rapid	Underestimating Unreliable
In vivo	Mass-balance	Inhalation Ingestion Dermal	Animals	Bioavailability factor	Accurate Reliable	Technically demanding Expensive Time-consuming
In vivo	Various techniques	Inhalation Ingestion Dermal	Animals	Dissolution rate (Prediction of bioavailability)	Simple Inexpensive Rapid	Not accurate Unreliable
In vitro	Various techniques	-	-	Same as above	Same as above	Same as above
In vivo	Measurement of exhaled air level	Inhalation	Humans Animals	Deposition fraction (gas/vapor and aerosol)	Simple Inexpensive Rapid	Not accurate (Overestimation of bioavailability factor in some cases)
In vivo	Various techniques	Inhalation	Humans Animals	Mechanical clearance fraction and retention fraction (Prediction of bioavailability factor)	Simple Inexpensive Noninvasive (In some cases)	Not accurate

(Continued)

Method	Technique	Administered route	Subject	Objective	Strength	Limitation
In vitro	Various techniques	-	-	Inhalable fraction Particle size Water solubility Blood/air or octanol/ water partition coefficients (Prediction of deposition fraction and bioavailability factor)	Simple Inexpensive	Not accurate
In vitro	Isolated perfused lung	Inhalation	Animals	Bioavailability factor	Accurate Remaining pulmonary function and structure integrity	Expensive Viable in short period of time Technically demanding
In vivo	Measurement of fecal level	Ingestion	Humans Animals	Bioavailability factor	Simple Convenient	Overestimation Unreliable
In vivo	Chronic isolated loop	Ingestion	Animals	Bioavailability factor	Controlling variables Remaining physiological function	Overestimation Unreliable
In vivo	Measurement of liver ratio	Ingestion	Animals	Bioavailability factor	Direct measurement of bioavailability factor	Only available by making an assumption that the levels of xenobiotics primarily reach inig the liver represent the systemic levels

(Continued)

Method	Technique	Administered route	Subject	Objective	Strength	Limitation
In vitro	Various techniques based on pH partition theory	-	-	Lipid/water or water/lipid partition coefficient in the gastrointestinal tract (various pH environment) (Prediction of bioavailability factor)	Simple Inexpensive Rapid	Not accurate
In vivo	Surface disappearance	Dermal	Humans Animals	Bioavailability factor	Simple Inexpensive Noninvasive (in some cases)	Not accurate (Overestimation)
In vitro	Diffusion cell	-	Skin from humans and animals	Bioavailability factor Permeability Diffusivity Partition coefficient	Simple Inexpensive Controlling the variables	Unreliable Unknown relationship between *in vivo* and *in vitro* for most xenobiotics
In vitro	Diffusion cell	-	Artificial membrane	Bioavailability factor Permeability Diffusivity Partition coefficient	Simple Inexpensive	Unreliable Not accurate

*: In the past, *in vivo* bioavailability studies of some xenobiotics were conducted in human volunteers but human study is not viable anymore.

5. Sources of Contaminants

5.1 Chromated Copper Arsenate (CCA)-based Mixtures

CCA is widely used for preserved wood foundation. CCA-based wood preserving mixtures were once utilized at two former wood preserving sites in Alberta (Hoffmann and Hrudey 1990). The working fluid, which is a yellow-green, water-soluble and acidic liquid, odorless and non-volatile, contains 1.5 to 4.0% of CCA (Environment Canada 1988). Water is a carrier. Chromium, arsenic and copper in CCA occur as oxides, such as CrO_3, CuO and As_2O_5. Generally, the environmental hazard from using the mixtures at the sites is due to their chemical release into the surrounding areas as drips and sludges (Hoffmann and Hrudey 1990). Drips may take place through leaking pipes and drippage during storage of treated timber. Sludges may arise from the retort sump or filters that recycle unused fluids. As a result, surface soils and lagoons at operating sites have been polluted by CCA fluid.

5.2 Pentachlorophenol(PCP)-based Mixtures

Pentachlorophenol(PCP)-based wood preserving mixtures were employed at four former wood preserving sites in Alberta (Hoffmann and Hrudey, 1990). The PCP mixtures consist of 3 to 6% technical-grade PCP with petroleum carriers, commonly, diesel fuel. Mixtures in technical-grade PCPs wood preserving solution typically comprise PCP, tetrachlorophenol, trichlorophenol, chlorinated phenoxyphenols, and some contaminants, such as polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The carrier contains a variety of organic components, e.g. benzene and toluene and metals, e.g. lead.

The physicochemical properties of PCP-based mixtures are similar to those of diesel fuel. Diesel fuel possesses a lower density and higher viscosity than water.

PCP-based mixtures were also discharged into the environment as drips and sludges at former wood preserving sites (Hoffmann and Hrudey 1990). The chemicals were likely to be retained in surface soils by capillary forces but they will move downward to groundwater. The physicochemical properties of PCP carrier solutions cause them to float on the groundwater surface. Soil-bound chemicals could also be suspended as airborne dusts if soils become desiccated.

5.3 Creosote-based Mixtures

Creosote-based wood preserving mixtures were used at one of the former wood preserving sites in Alberta (Hoffmann and Hrudey 1990). The coal-tar creosote working mixtures consist of several thousand compounds in addition to petroleum carriers. The main compounds in the mixtures that produce concerns for adverse health effects are carcinogenic polynuclear aromatic hydrocarbons (PAHs), phenolics (phenol and various alkyl-substituted phenol) and a wide range of heterocycles (e.g. aniline, quinoline, benzothiophene and dibenzothiophene).

Creosote is a heavy, oily liquid with a low water solubility. Creosote-based wood preserving mixtures were also discharged into the environment as drips and sludges at former wood preserving sites (Hoffmann and Hrudey 1990). Based on various physicochemical properties for the chemicals, these compounds released to the environment may partitioned into air, surface water, groundwater and soil. PAHs, in particular, can persist in soil for a long periods.

6. Review of Exposure Routes and Bioavailability Factor for Each Contaminant

6.1 Chromium and Chromium Compounds

Chromium (Cr) is an element commonly found on earth. This element exists naturally in the elemental form and oxidation states ranging from -2 to +6 (NAS 1974, USEPA 1985a, 1990b, Lide 1993). However, only elemental chromium, chromium (III) and chromium (VI) forms are stable and biologically important (Hartford 1979, USEPA 1984a, Hathaway *et al.* 1991, ACGIH 1991).

Because of its widespread distribution, chromium is commonly detected in soil, water, food and air. Chromium (VI) compounds, e.g. $Cr_2O_7^{-2}$ and CrO_4^{-2}, are the primary forms of atmospheric chromium that are mobile, reactive and bioavailable (USEPA 1984a). Both chromium (III) and chromium (VI) are stable in water under thermodynamic equilibrium and in sandy soils containing low level of organic matter (Bartlett and James 1988, Bartlett 1991, USEPA 1990b). These two forms of chromium are likely to be interconvertible in natural water and in soils, a process called chromium cycling (Roberts and Anderson 1975, Schroeder and Lee 1975, Eary and Rai 1987, 1988, Rai *et al.* 1989, Bartlett 1991). Chromium compounds in airborne particulates can deposit on the plant surface, and be taken up by plant roots growing on contaminated soils (NAS 1974). Chromium content in foodstuff can vary considerably. In general, the chromium content of most foods is very low (NAS 1974, IARC 1980). Larger quantities of chromium have been reported in meats, vegetables, unrefined sugar and crustaceans in comparison with fish and fruit (NAS 1974).

Chromium (III) is considered to be an essential trace nutrient for humans (Mertz 1975, Anderson 1981, 1989, USEPA 1984a, WHO 1988, Iyengar 1989, ATSDR 1989a, Burg and Liu 1993). However, recent evidence has revealed that chromium (III) compounds could slowly react with the nucleic acids and proteins *in vitro* or *in vivo*, thereby being mutagenic and genotoxic (Cupo and Wetterhahn 1985, Elias *et al.* 1986, Sugden *et al.* 1990, Snow 1991). Chromium (VI), mainly produced by industrial processes, has been classified as a human carcinogen by inhalation. Specifically, exposure to certain water-insoluble chromium (VI) compounds, particularly industrial chromate production was found to be associated with the lung cancer in humans (IARC 1980, 1987, 1990, USEPA 1984a, WHO 1988, Cohen *et al.* 1993). Data reviewed from IARC (1980) also indicate that a few water-soluble chromium (VI) compounds (chromium trioxide) and some water-insoluble compounds (calcium chromate, lead chromate, and zinc chromate) may be related to an increased risk of lung cancer in animals. Evidence on the carcinogenicity of chromium compounds via ingestion and dermal exposure is lacking.

Water-soluble chromium compounds can be rapidly absorbed in the respiratory system. They are slightly bioavailable in the gastrointestinal system. Quantitative data on skin absorption are lacking.

6.1.1 Exposure Routes and Receptors

For occupational exposure, inhalation and dermal contact are all primary routes (WHO 1988). Exposure is often due to both chromium (VI) and chromium (III). Permissible exposure limits in air are 0.5 mg/m^3 for chromium (III) and 0.05 mg/m^3 for chromium (VI) at the workplace (Carson *et al.* 1986, Sittig 1991). Inhaled water-soluble chromium compounds can readily penetrate through the air-blood barrier. In contrast, water insoluble ones can remain in the respiratory tract. Workers may contact chromium products through skin.

The general population is mainly exposed to chromium compounds by ingestion of food, water or soil containing chromium. The levels of chromium compounds in foodstuff and drinking water are generally very low, and they are only slightly absorbed in the gastrointestinal tract. Thus, the ingestion route from diet and drinking water is generally considered to be of little significance for most humans.

The permissible exposure limit for chromium in drinking water is 50$\mu g/L$ (MSSC 1989). Concentrations of chromium (VI) in groundwater are rarely detected. This is due to the common reduction of chromium (VI) to (III) when these compounds leach from soil into groundwater. Accordingly, the potential hazard from groundwater appears to be negligible. On the other hand, the residents living near industrial and contaminated areas may be exposed to higher levels of chromium (VI) (USEPA 1984a). Under this circumstance, inhalation may become significant for human exposure. For example, a study from Hudson County, New Jersey, where there was soil contaminated with chromium processing waste, indicated elevated chromium concentrations in outdoor air, indoor air and house dust (Lioy *et al.* 1992). In particular, chromium in household dust was considered to be the primary source of household exposure. The waste was used at numerous locations within the County as fill. Consequently, chromium (VI) and chromium (III) were present at the surface at many of these locations. These locations represented a significant source of emission contributing to the elevated chromium levels in air and house dust.

Soil ingestion is a potential exposure route for young children with pica behavior living near chromium-contaminated sites. But the hazard posed by soil ingestion is minimal unless the concentrations of soil chromium are extremely high. The safe levels for soil chromium suggested by the New Jersey Department of Environmental Protection were less than the 1000 ppm for total chromium and 75 ppm for chromium (VI) (Paustenbach *et al*. 1991a). Occasionally, people may contact contaminated soil or water on their skin during outdoor activities. To date, however, no special groups with an exceptional risk of exposure outside specific occupational environments have been identified (USEPA 1984a).

6.1.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.1.3 on Bioavailability.

Distribution

Chromium compounds absorbed (except chromates) are rapidly removed from the systemic circulation, distributed in various tissues, or eliminated via urine (Mertz 1969, WHO 1988). In humans, chromium and its compounds following inhalation are mainly distributed in the lung, lymph nodes, kidney, liver, bladder and bone (IARC 1980). By ingestion, the high chromium levels were found in hilar lymph nodes, lung, spleen, liver, kidney and heart (Teraoka 1981).

Metabolism and Excretion

Chromium (VI) is found to be reduced to chromium (III) by *in vivo* and *in vitro* studies (Wiegand 1984). Chromium (VI) compounds readily enter red blood cells by simple diffusion, where it is strongly bound to hemoglobin, while chromium (III) compounds have limited ability to cross blood cell membranes and have an affinity for serum protein (Mertz 1969, Seiler and Sigel 1988).

Excretion of chromium from the human body, by inhalation and ingestion, primarily occurs via urine. In most cases, chromium (VI) compounds are rarely detected in the urine because of the reduction of chromium (VI) to chromium (III) in biological systems or the formation of chromium protein complexes (USEPA 1984a). Chromium (III) is excreted to a lesser degree compared with chromium (VI) because chromium (III) tends to form a less excretable form, hydrate complexes. Data on excretion specifically following dermal exposure have not been located.

Following the administration of intravenous chromium (VI) and (III) compounds, there was about 3.5% to 8.4% of chromium (VI) and 0.1% to 0.5% of chromium (III) in the bile in rats (ATSDR 1989a).

6.1.3 Bioavailability

A. Inhalation

Humans

Several studies have revealed that chromium (VI) and (III) compounds could be absorbed following inhalation exposure in the occupational or general population, as evidenced by increased chromium in the blood and urine (Baetjer *et al.* 1959, NAS 1974, Gylseth *et al.* 1977, Cavalleri and Minoia 1985, Randall and Gibson 1987, Stern 1992). The ICRP Task Group (Morrow *et al.* 1966) classified the oxides and hydroxides of chromium as a slow clearance group, and other chromium compounds as a fast clearance group. Consequently, water-insoluble compounds tend to remain in the respiratory tract, while water-soluble compounds are likely to be absorbed in various regions of the respiratory tract. Inhaled water-soluble chromium(VI) compounds are rapidly absorbed according to studies on biological monitoring of occupational exposures (Franchini *et al.* 1984). However, quantitative data on the deposition, retention and clearance of chromium compounds for humans are generally lacking.

Animals

Once water-insoluble chromium (III) aerosols are inhaled, they are primarily cleared by mechanical clearance processes. One study from Visek *et al.* (1953) reported that chromic chloride ($CrCl_3$) dust administered by intratracheal instillation in rats was primarily excreted in the feces (55%). Their results indicated that a large amount of inhaled chromium (III) was ultimately swallowed into the gastrointestinal tract. In another study (Sanders *et al.* 1971), chromic oxide (Cr_2O_3) dust was introduced to hamsters by an inhalation chamber for 4 hours at dose of 0.5 to 1.0 mg/m^3. Over 90% of chromium (III) particles with the size of 0.33 μm count median diameter was ingested by pulmonary macrophages.

Absorption of chromium (III) from the respiratory tract is very low. Three studies showed that the bioavailability factors were less than 10% in small rodents following the intratracheal administration of water-soluble chromium chloride at a single dose ranging from 100 to 200 μg by using a mass-balance technique (Baetjer *et al.* 1959, Visek *et al.* 1953, Wiegand *et al.* 1984). The majority of the compounds inhaled were eliminated by mechanical clearance into the gastrointestinal tract. Baetjer *et al.* (1959) reported only 4% of administered chromium in the blood and other tissues and 69% in the lung at ten minutes post-exposure. At 24 hours, there was 6% present in the urine and 45% remaining in the lung. In this case, the cumulative urinary value accounted for the absorption fraction from both the respiratory tract and gastrointestinal tract. In rats, the respiratory bioavailability factor was found to be less than 5% (Visek *et al.*, 1953). Rabbit experiments indicated that 9% of chromium (VI) compounds could be absorbed from the lung after 4 hours dosing (Wiegand *et al.* 1984).

Water-soluble chromium (VI) compounds are more readily absorbed than chromium (III) compounds. In the experiments reported by Baetjer *et al.* (1959), 15% of potassium dichromate (CrVI), after ten minutes exposure, remained in the lung, with 20% present in the red blood cells, and 5% in the liver. Overall, the bioavailability factor of chromium (VI) was approximately 29%. In rabbits, the overall bioavailability factor was 46% after 4 hours dosing (Wiegand *et al.* 1984).

Weber (1983) used the whole body or lung counting and mass-balance techniques to determine the absorption and distribution of a chromium (VI) compound (sodium dichromate) in rats. Following the intratracheal administration of the compound (50 μg), the retention fraction in the lung was 43%, 36%, and 12% at 6 hours, 24 hours and 40 days, respectively. Most of the compound within the lung was localized in type II alveolar cells which are part of the air-blood barrier. They can synthesize and release the surfactant to regulate the surface tension for alveoli. However, the lack of data on the urinary excretion precludes estimating the bioavailability factor from this study.

Bragt and his co-workers (Bragt and Van Dura 1983, Bragt *et al.* 1986) found that the clearance of chromates (sodium, zinc or lead chromate dusts) from the lung and blood was directly associated with the degree of water solubility of the salts. 20% of sodium chromate (water-soluble) and 26% of zinc chromate (less water-soluble) were absorbed from the lung during 10 days exposure. Lead chromate (water-insoluble) was only slightly bioavailable (2%).

Zinc chromate (VI) dust was reported to be rapidly absorbed in rats (Langård *et al.* 1978). The dust contained 20.4% chromium (VI), with 76% as an inhalable fraction. After 100 minutes inhalation exposure with a dose of 7.35 mg/m^3 in an inhalation chamber, the blood chromium levels were increased 5-fold. However, the compound was slowly eliminated from the blood.

Chromium mixtures administered by inhalation generally have a low bioavailability (Salem and Katz 1989). Whetlerite dust (2.02% total chromium, 0.98% insoluble chromium (VI), 0.30% soluble chromium (VI) and 0.74% insoluble chromium (III) was intratracheally introduced to rats at a single dose of 320 µg/g. After 24-48 hours following dosing, only 17% of the total chromium dose was recovered in the lung, liver, kidney, spleen, and blood.

B. Ingestion

Humans

Chromium (III) compounds in diet, water or tablets are only slightly bioavailable in humans. Doisy *et al.* (1968) examined a number of normal, elderly and diabetic subjects who ingested chromic chloride at 150 µg/day with meals. After three days exposure, only 0.69% chromium was taken up by normal subjects, and 0.92% in elderly subjects. Donaldson and Barreras (1966) reported that the absorption fraction of chromic chloride in water for fasting subjects was 0.4% according to the fecal excretion values at 6 days after dosing 20 ng chromium. In another study, 0.4% of chromium was found in the urine following the administration of 200 µg/day chromium chloride tablets (Anderson *et al.* 1983). Offenbacher *et al.* (1986) studied two subjects who ingested chromium at 37 µg/d. They observed that the average absorption fraction was 1.8%. Another study found that the intake of low diet chromium (10 µg/7 days) had a high absorption (2%), while the intake of higher diet chromium had a lower absorption fraction (0.5%) (Anderson and Kozlovsky 1985).

In general, chromium (VI) compounds appear to be absorbed more rapidly and readily. A study from Donaldson and Barreras (1966) showed a

10.6% bioavailability factor for fasting subjects who were orally administered 20 ng of sodium chromate.

Animals

Data from animal experiments revealed that the degree of absorption of chromium (III) compounds was also low. Rats fed by chromic chloride in a buffer solution only absorbed less than 0.5% of chromium compounds after 4 days exposure (Visek *et al.* 1953). With the administration of chromic chloride by gavage, the bioavailability factor in rats was 2% to 3% (Mertz *et al.* 1965). Sullivan *et al.* (1984) observed that the extent of absorption of chromic chloride was higher in 2-day old rats (1.2%) than in adult rats (0.1%). The data on chromium (VI) absorption in rats and mice showed that bioavailability factors were slightly higher (2.3% to 2.4%) than that of chromium (III) compounds (Ogawa *et al.* 1976, Donaldson and Barreras 1966). Moreover, mice in fasting state could absorb more chromium (III) and (VI) (11%) than mice with a normal diet (Ogawa *et al.* 1976).

Bioavailability of plant chromium complexes was studied by Starich and Blincoe (1983). In their experiment, alfalfa and brewer's yeast were exposed to either chromium (III) or (VI) (CrO_4^{-2}) *in vitro*. Then, the plant chromium complexes were fed to rats for ten days. The bioavailability factors of plant chromium complexes were 35% for alfalfa seedings, 37% for alfalfa extract and 29% brewer's yeast. Other studies suggested that 10% to 25% of the Brewer's yeast chromium was bioavailable (Underwood 1977).

Other

In a soil solubility study, less than 0.5% to 1% of total soil chromium could be dissolved in an acid solution (pH 2 and pH 5 within 24 hours) (Paustenbach *et al.* 1991a, Sheehan *et al.* 1991). As a result, only very a small fraction of soil chromium was judged to be available for absorption in the gastrointestinal tract.

C. Dermal

Some of investigations indicated the absorption of chromium (III) and (VI) through the skin in humans (Samitz and Shrager 1966, Baranowska-Duttiewicz 1981a, Kelly *et al.* 1982, Lindberg and Vesterberg 1983, Randall and Gibson 1987, Gammelgaard *et al.* 1992). In principle, chromium VI compounds can easily pass through the skin without the formation of complexes (Bagdon and Hazen 1991). Chromium VI compounds can also be converted to chromium (III) compounds at certain applied concentrations (Gammelgaard *et al.* 1992). In contrast, chromium (III) compounds are likely to bind non-specific protein on the skin with resulting difficulty in penetration of the skin. Samitz and Shrager (1966) reported that chromium (III) nitrate only slightly penetrated the intact human skin but chromium (III) sulfate failed to pass through the skin.

The dermal permeability of chromium (III) and (VI) compounds has been studied in humans *in vivo* and *in vitro*. In one *in vivo* study, Na_2CrO_4 (Cr VI) solution at different concentrations (0.01M, 0.1M and 0.2M) was applied on the forearm skin in human volunteers (Baranowska-Dutkiewicz, 1981a). The dermal permeability was 3.5×10^{-3} cm/h at 0.01M level after 15 minutes dosing and 1×10^{-3} cm/h at 0.2 M after 60 minutes dosing. The dermal permeability of chromium (III) and (VI) compounds through human skin *in vitro* is lower than that *in vivo*. Gammelgaard *et al.* (1992) reported only 0.01×10^{-3} cm/h of *in vitro* dermal permeability for a chromium (VI) compound ($K_2Cr_2O_7$) in humans.

In animal experiments, absorption of chromium compounds varied with the concentrations of the compounds at the applied site (Wahlberg and Skog 1965). Chromic chloride and sodium chromate ranging from 0.00048 to 1.689 molar were applied to the skin of guinea-pigs. In the lowest (0.00048 and 0.005 molar) and highest (0.753 and 1.689 molar) concentrations, the absorption fractions were less than 1% after 5 hours exposure. For the remaining concentrations (0.017 to 0.398 molar), the absorption fractions of chromium (VI) were higher than chromium (III).

Dermal absorption of chromium is pH dependent. With a rising pH of applied solution, the extent of absorption of chromium (III) and (VI) compound will increase. Czernielewski *et al.* (1965) observed that guinea-pigs with pretreatment of alkali on the skin had a higher degree of absorption of chromium (VI) (12.5%) than those without alkali treatment (1.30%).

When chromium compounds are bound to soil, the dermal bioavailability will decrease. Based on a solubility study, an estimated 1% of total soil chromium may leach from the soil matrix and be dissolved in sweat on the skin (Paustenbach *et al.* 1991b).

D. Summary

Overall, either chromium (III) or chromium (VI) is bioavailable in humans and animals through inhalation, ingestion and dermal exposure

(Table 6-1, 6-2, 6-3 and 6-4). Available quantitative results are summarized in Figure 6-1. The extent of absorption is higher via inhalation than via other two routes. Chromium (VI) (other than water-insoluble compounds) is more readily absorbed than chromium (III) by these three routes. Species differences are significant but small.

6.1.4 Uncertainties

At contaminated sites, chromium (VI) compounds (CrO_3) may be present in surface soil and lagoons. Soil-bound chromium may be transported off-site to residential areas by wind. As a result, residents in the vicinity of chromium contaminated sites might be exposed to the airborne chromium by inhalation.

The respiratory tract has been recognized the only target tissue where the carcinogenicity arises via inhalation of chromium (VI). However, the mechanisms of chromium carcinogenesis have not been clarified. Some studies indicated that the occurrence of the lung cancer was related to the genotoxicity in alveolar tissue that was contributed by the reduced forms (Cr III or Cr V) of chromium (VI) (USEPA 1990c). Therefore, an understanding of not only the extent of absorption but also deposition, retention and mechanical clearance of chromium-containing aerosols are important in cancer risk assessment. Factors, such as particle size, inhalable fraction and solubility of soil chromium in the respiratory tract (under neutral pH conditions), strongly influence these processes. These parameters at former wood preserving sites have not been characterized. To date, the respiratory bioavailability has only been available for some water-soluble and a few water-insoluble chromium (VI) compounds in animals. Extrapolation of chromium (VI) bioavailability from animals to humans carries uncertainty.

The quantitative data for CrO_3 are lacking. However, CrO_3 is highly water-soluble and reactive. It might leach from soil particles trapped in the respiratory tract and be rapidly absorbed into the systemic circulation in humans following inhalation.

Chromium (VI) can leach from the surface soil into groundwater or be released into the surface water by runoff and fallout. Suspended chromium dust may deposit on the local grown plants. The levels of chromium (VI) in drinking water and local grown plants are not available at the contaminated sites. However, chromium (VI) is readily reduced to chromium (III) in water and plants. Consequently, resident exposure to chromium (VI) from drinking water and local grown plants should be negligible.

At operating sites, the levels of soil-bound chromium (VI) may be high. But the levels of these compounds in residential areas should be lower. Residents, particularly children with pica behaviour, may be exposed to soil chromium by ingestion. In fact, the oxidizing potential of chromium (VI) ions relies upon pH. In the acidic environment (e.g. gastric juice), chromium (VI) is very efficiently reduced to chromium (III) that is less readily absorbed in the gastrointestinal tract (Petrilli and De Flora 1988). Thus, human gastric juice is considered to be a significant barrier against the absorption of chromium (VI) from the gastrointestinal tract. Furthermore, no evidence currently supports the carcinogenicity of chromium (VI) by ingestion. Therefore, the health hazard by soil ingestion should be minimal.

Oral bioavailability in humans and animals is well studied. Chromium (III) is likely to be absorbed in the small intestine by passive diffusion (Dowling *et al.* 1989). The mechanism of absorption for chromium (VI) in the gastrointestinal tract is poorly understood.

Because the majority of chromium compounds absorbed are eliminated via urine, some results of oral bioavailability for chromium are derived from chromium urinary excretion data, by making an assumption that the urinary excretion is equal to total absorption by ingestion (Franchini *et al.* 1984, WHO 1988). However, quantitative study on distribution of chromium is currently incomplete. In particular, chromium (VI) absorbed effectively passes through cell membranes in the body, and may be partially and slowly excreted via urine. Thus, chromium urinary data represent a minimum chromium absorption potential but probably not recent exposure (USEPA 1990c). The values from fecal excretion generally reflect the net absorption because (1) the unabsorbed chromium accounts for most of total chromium excretion in feces; (2) the biliary excretion of chromium accounts for a very small part in feces; and (3) hepatic first-pass effect is not observed for chromium.

Oral bioavailability values are derived from the water-soluble compounds contained in water and diet. However, quantitative data for water-insoluble or soil chromium (VI) are not available. Solubility studies reveal a very low solubility rate for soil-bound chromium (VI) in an acidic environment.

In animal studies, plant chromium complexes formed *in vitro* are highly absorbed but these levels of plant complexes do not reflect realistic plant intake levels from the environment.

Dermal exposure for residents near the contaminated sites likely represents a minor route. Residents will only occasionally contact soil-bound or waterborne chromium (VI) with their skin. Furthermore, soil chromium (VI) is not efficiently dissolved in sweat on skin (1%). Likewise, the dermal bioavailability factors in animals have been shown to be low. Quantitative values in humans are limited. Finally, there is no evidence indicating the carcinogenicity of chromium (VI) through dermal exposure.

The general population is often exposed to chromium mixtures in the environment. The information on the interaction between chromium and other xenobiotics during absorption is limited.

6.1.5 Conclusions

In an occupational setting, inhalation and dermal exposure are major exposure routes for chromium. For the general population, ingestion of foodborne, waterborne and soil-bound chromium compounds is a primary exposure route. In most cases, however, this route is less significant because of the low contents of chromium in these media and low bioavailability in the gastrointestinal tract. Residents living near heavily chromium contaminated surface soils have some potential to be exposed to these compounds by inhalation, dermal contact or soil ingestion. These surface soils may represent a source of chromium contamination for off site release and human exposure.

Both chromium (VI) and (III) are bioavailable to humans and animals. Chromium (VI) is more readily absorbed than chromium (III). In accordance with the carcinogenic risk by inhalation and toxic effects mainly from chromium (VI), the evaluation of chromium (VI) bioavailability is important.

Bioavailability factors for water-soluble chromium (VI) by ingestion are approximately 11% in fasting humans. Quantitative bioavailability factors by inhalation and dermal exposure for humans are not yet available.

Respiratory bioavailability factors from animal studies with small rodents are 20% to 47% of for water-soluble chromium (VI) and 2% for water-insoluble chromium (VI). Only 4% to 9% of chromium (III) can be absorbed from the lung. The oral bioavailability of chromium (VI) is about 2% in small rodents and 11% in fasting mice. The oral bioavailability of chromium (III) ranges from <1% to 2%. The values by dermal exposure to chromium (VI) vary with the concentrations of the compounds applied on the skin, ranging from 1.3% to 4% in small rodents. With alkali treatment of the skin, the dermal bioavailability increases to 12.5%. The dermal bioavailability of chromium (III) is about 2%.

The solubility of soil chromium (VI) is 0.5% to 1% in acidic condition (pH2 and pH5).

Based on the pH-dependent profile involved in chromium absorption, it is possible to reduce the extent of absorption of chromium through alteration of pH in chromium contaminated media and biological fluids in the human body.

Chromium carcinogenicity in the respiratory tract is currently considered to be a local effect. Thus, estimation of an internal dose for chromium cancer risk assessment is questionable.

6.1.6 Recommendations for Further Evaluation and Research

Evaluation of carcinogenic risk by inhalation of chromium (VI) compounds (including CrO_3) in humans and animals requires more knowledge on parameters relevant to estimating the respiratory bioavailability of chromium (VI) at specific sites. These include the respiratory bioavailability factor for each specific chromium form, fractions of deposition, retention and clearance, and physicochemical properties of airborne chromium at the sites, such as particle size distribution in air, inhalable fraction, and dissolution rate of chromium particulates in the respiratory tract (pH 7.4 environment). Clarifying the mechanisms of chromium carcinogenesis and determine the biologically active chromium will aid in assessing the potential risk for human arising from exposure to chromium.

Oral bioavailability of chromium (VI) in humans and animals has been well-documented. But the values from measurement of chromium urinary excretion are not reliable. Quantitatively studying the distribution of chromium will reduce the errors arising from this method.

Data on the levels of chromium (VI) in surface soil, groundwater, and local grown plants at specific sites are required for accurately assessing exposure to the general population because residents may be exposed to high level chromium (VI).

Bioavailability studies indicate that absorption of chromium via main exposure routes greatly depends on valence, water solubility, pH condition and chromium speciation. For quantitative risk assessment, it is necessary to determine valence states, chemical forms and water solubility of chromium compounds at chromium contaminated sites. The values of bioavailability and toxicity that are selected for calculating average daily doses, if possible, should be consistent with or close to those from chromium contaminated sites. For instance, the bioavailability factors for water-soluble chromium (VI) may be used for CrO_3.

With exposure to chromium mixtures, the interaction between chromium and other xenobiotics may alter the absorption profiles of chromium. Thus, studies on the bioavailability for chromium mixtures are critical.

Table 6-1 Respiratory Bioavailability of Chromium in Animals

							Cr Recovery						
Compound	Species	Administered method	Cr dosage	Experimental method	Exposure duration	Cr Retention in the lung	Blood	Urine	Feces	other	Total clearance	BF[1]	Ref.
Cr(III)													
$CrCl_3$	Guinea-pig	Intra-tracheal	200 µg	Mass-balance	10 min 24 hr 60 d	69% 45% 12%	4	6%			31% 55% 88%	4%[2] 6%	a
$CrCl_3$	Rat	Intra-tracheal	40-250 µg	Mass-balance	7 d			7%	55%			<5%	b
$CrCl_3$	Rabbit	Intra-tracheal	100 µg	Mass-balance	4 hr	85%	0.83%	7.9%		0.51%	15%	9%	c
Cr(VI)													
$K_2Cr_2O_7$	Guinea-pig	Intra-tracheal	200 µg	Mass-balance	10 min 24 hr 60 d	15% 11% 1.6%	20% 9%	13%		5% 7%	85% 89% 98%	25%[2] 29%	a
$Na_2Cr_2O_7$ $+Na_2CrO_4$	Rabbit	Intra-tracheal	100-500 µg	Mass-balance	4 hr	46.8%	6.1%	15.5%		24%	53%	47%	c
Na_2CrO_4 $ZnCrO_4$ $PbCrO_4$	Rat	Intra-tracheal	69 µg 66 µg 38 µg	Mass-balance	10 d		0.25% 0.75% 0%	20% 25% 2%	20% 50% 75%			20% 26% 2%	d

(1)BF = Bioavailability Factor (2) the value from blood and other tissues.
Adapted from a: Baetjer *et al.* 1959; Bb Visek *et al.* 1953; c: Wiegand *et al.* 1984; d: Bragt and van Dur, 1983

Table 6-2 Oral Bioavailability of Chromium in Humans

Compound	Number of Subjects	Chromium carrier	Chromium dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Cr(III)							
$CrCl_3$	15 (normal) 7 (Elderly)	Meal	150 μg/d	Urinary excretion	72 hr	0.69%(0.6-1.3%) 0.92%(0.4-1.5%)	a
$CrCl_3$	42	Tablet	200 μg/d	Urinary excretion	24 hr	0.4%	b
$CrCl_3$	6 (Fasting)	Water	Single: 20 ng	Urinary and fecal excretion	6 d	0.4% (Minimal 0.5%)	c
Dietary chromium	2	Diet	37 μg/d	Urinary and fecal excretion	6 d	1.8% (Minimal 0.8%)	d
Dietary chromium	32	Diet	10-40 μg/d	Urinary excretion	7 d	2%(10μg) 0.5%(40μg)	e
Cr(VI)							
Na_2CrO_4	6 (Fasting)	Water	Single: 20 ng	Urinary and fecal excretion	6 d	10.6% (Minimal 2.1%)	c

Adapted from a: Doisy *et al.* 1968; b: Anderson *et al.* 1983; c: Donaldson and Barrera, 1966; d: Offenbacher *et al.* 1986; e: Anderson and Kozlovesky 1985

Table 6-3 Oral Bioavailability of Chromium in Animals

Compound	Species	Administration methods	Chromium carrier	Chromium cosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Cr(III)								
$CrCl_3$	Rat	Feed	Buffer solution	1.25-2.5 mg	Fecal excretion	4 d	<0.5%	a
$CrCl_3$	Rat	Gavage	Acidic solution	Single: 0.15-10 µg/100g	Whole-body counting	4-10 d	2-3%	b
$CrCl_3$	Rat	Gavage	Saline	Single: 1 ng	Urinary and fecal excretion	7 d	2% (Minimal 1.4%)	c
$CrCl_3$	Mouse	Oral	-	-	Whole-body counting; urinary and fecal excretion	48 hr	1.4%	d
$CrCl_3$	Mouse (fasting)	Oral	-	-	Whole-body counting; urinary and fecal excretion	48 hr	11%	d
$CrCl_3$	Rat (adults)	Gavage	Acidic solution	1 µc	Whole-body counting	–	0.1%	e
$CrCl_3$	Rat (2-day old)	Gavage	Acidic solution	1 µc	Whole-body counting	–	1.2%	e

Adapted from a: Visek *et al.* 1953; b: Mertz *et al.* 1965; c: Donaldson and Barreras 1966; d: Ogawa *et al.* 1976; e: Sullivan *et al.* 1984;

(Continued)

Compound	Species	Administration methods	Chromium carrier	Chromium dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Cr(VI)								
Na_2CrO_4	Rat	Gavage	Saline	Single: 1 ng	Urinary and fecal excretion	7 d	2.3% (minimal 0.8%)	c
Na_2CrO_4	Mouse	Oral	-	-	Whole-body counting; urinary and fecal excretion	48 hr	2.4%	d
Na_2CrO_4	Mouse (fasting)	Oral	-	-	Whole-body counting; urinary and fecal excretion	48 hr	11%	d
Mixtures								
Chromium complex	Rat	Feed	*Alfalfa*: seedings labeled extract *Brewer's yeast*	-	Urinary and fecal excretion	10 d	35% 37% 30%	f

Adapted from f: Starich and Blincoe 1983

Table 6-4 Dermal Bioavailability of Chromium in Animals

Compound	Species	Administration methods	Chromium carrier	Chromium concentration	Experimental method	Exposure duration	Bioavailability factor	Ref.
Cr(III)								
$CrCl_3$	Guinea-pig	Skin	Water	0.017M	External counting	5 hr	2.2%	a
				0.080M			1.6%	
				0.126M			1.8%	
				0.239M			2.0%	
Cr(VI)								
Na_2CrO_4	Guinea-pig	Skin	Water	0.017M	External counting	5 hr	2.6%	a
				0.080M			2.1%	
				0.239M			2.3%	
				0.261M			4.0%	
				0.398M			2.8%	
Na_2CrO_4	Guinea-pig	Dorsal area	Saline	15 µg	Mass-balance	24 hr	1.3% 12.5%(pretreated alkali)	b

Adapted from a: Wahlberg and Skog 1965; b: Czernielewski *et al*. 1965

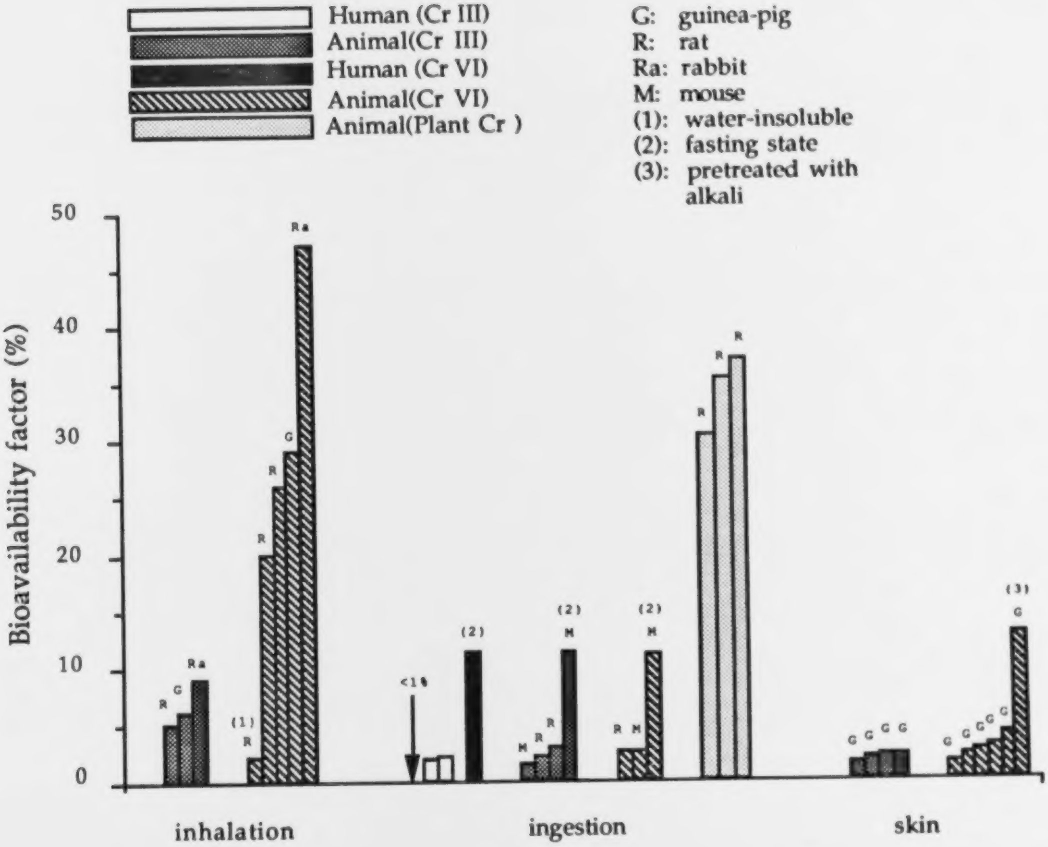

Figure 6-1 Bioavailability factors of chromium compounds in humans and animals by three exposure routes[Adapted from sources in Table 6-1: a,b,c,d, (inhalation); Table 6-2: a,b,c,d,e (ingestion); Table 6-3: b,c,d,f(ingestion); and Table 6-4: a,b (skin)]

6.2 Arsenic

Arsenic is an element commonly found on earth. Common oxidation states include 0, -3, +3 and +5 for inorganic and organic forms (NAS 1977a, USEPA 1984b, Lide 1993).

Arsenic is frequently released to the air, soil, water and biota. Arsenic in particulate matter may occur in inorganic and organic forms (WHO 1981). Arsenic (III) in the air is readily oxidized to arsenic (V). The oxidized arsenic is relatively stable in the air (NAS 1977a). Both inorganic and organic compounds are also present in surface water. Arsenic (V) usually predominates in surface water. Arsenic apparently accumulates in soils with inorganic form, complexing and chelating by organic material, iron, aluminum and calcium (ATSDR 1989b, 1993a). Waterborne and soil-bound arsenic readily undergo a series of transformations, such as oxidation/reduction, methylation by microorganisms, volatilization, leaching, adsorption and precipitation (NRCC 1978, Woolson 1983, ATSDR 1989b, 1993a). Plants rarely accumulate high concentrations of arsenic because this element is phytotoxic. Plants tolerant to high levels of soil arsenic include potatoes, tomatoes, cabbage, carrots, grapes, Sudan grass, and corns (Walsh *et al.* 1977). Roots usually concentrate higher arsenic than leaves. Bioaccumulation of arsenic takes place in food chains, particularly in aquatic organisms. Data from the food monitoring programs in the U.S. indicate that most arsenic was present in the meat-fish-poultry class of the total diet (Jelinek and Corneliussen 1977).

All soluble arsenic compounds appear to have toxic effects to humans. There is sufficient evidence to indicate an association between lung or skin cancer and respiratory or oral exposure to inorganic arsenic. In humans, occupational exposure to inorganic arsenic, particularly in metal smelting operations and pesticide production, is primarily associated with an increase of the incidence of lung cancer (Ott *et al.* 1974, Mabuchi *et al.* 1979, IARC 1980, 1987, WHO 1981, USEPA 1984b, Enterline *et al.* 1987, Mass 1992). The active arsenic species linked to the lung cancer is not clear but it is possibly related to the refining of As_2O_3 (Tinwell *et al.* 1991). Inorganic arsenic in animals has not been found to be carcinogenic (IARC 1987).

There is also sufficient evidence of a relationship between the development of skin cancer and chronic oral exposure to inorganic arsenic (potassium arsenite) in drinking water (Tseng *et al.* 1968, Tseng 1977, IARC

1980, 1987, USEPA 1984b, Mass 1992). Some studies suggested that a variety of internal tumors (bladder, kidney, liver and leukemia) may also be related to oral exposure to inorganic arsenic (ATSDR 1989b, 1993a, Bates *et al.* 1992, Mass 1992, Smith *et al.* 1992). To date, the carcinogenesis via dermal exposure has not been observed (ATSDR 1989b, 1993a).

In the respiratory tract and gastrointestinal tract, water-soluble inorganic or organic arsenic compounds appear to be highly bioavailable.

6.2.1 Exposure Routes and Receptors

Humans may be routinely exposed to arsenic at low levels by inhalation, ingestion and dermal route because of the extensive distribution of arsenic in the environmental media.

In an occupational setting, especially in metal smelters and arsenical pesticide manufacture, there are high levels of airborne arsenic (as much as 20 $\mu g/m^3$) (Vahter 1988). Inhalation of these compounds is a primary exposure route for workers. Also, workers may accidently contact aqueous solutions of inorganic arsenic with their skin.

The general population exposure to arsenic mainly results from ingestion of drinking water, food and drugs. Residents living near industrial areas may be subject to arsenic exposure by inhalation, ingestion and dermal contact.

Arsenic concentrations in drinking water vary extensively with geographic regions. In some areas, high levels of arsenic in drinking water have been observed (WHO 1981). With the presence of inorganic arsenic, the oral exposure to the contaminated drinking water may pose a significant health risk to humans (Abernathy and Ohanian 1992).

A major arsenic source for the general population via ingestion comes from daily food, in particular, seafood that binds some organic arsenic compounds. With the exception of seafood, arsenic concentrations in general daily food are very low (Jelinek and Corneliussen 1977). Moreover, organic arsenic forms present in most foods are readily eliminated in an unchanged form from the human body and seldom accumulate to toxic levels (Foa *et al.* 1984). Organic arsenic in fish (i.e. fish arsenic) generally has a low toxicity to humans. As a result, the potential hazard by consumption of food might be insignificant (Abernathy and Ohanian 1992). Occasionally, the subgroup of population who excessively consume seafood or local grown vegetables containing a large amount of inorganic arsenic might be at high risk (WHO 1981, Vahter 1988).

In a few of cases, residents living near contaminated areas may be exposed to arsenic by ingestion of soil or dust. Typically, the soil-bound arsenic is unlikely to accumulate to a high level. Exposure by ingestion of soil and dust may rarely be significant. Dermal exposure through contact with soil is likely to be minor because of the very low bioavailability (USEPA 1984b).

Generally, the inhalation exposure route is insignificant for the general population unless people inhale excessively high levels of airborne arsenic from contaminated areas (Murphy *et al*. 1989).

Additionally, the determination of exposure routes depends on the population sensitivity (ATSDR 1989b, 1993a). According to the metabolism process in which inorganic arsenic is detoxified in the liver by enzymic methylation, different degrees of enzymic activity in individuals strongly affects the toxic effects of arsenic to humans.

6.2.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.2.3 on Bioavailability.

Distribution

Once absorbed, inorganic arsenic is initially bound to the globin portion of hemoglobin in the red blood cells (Malachowski 1990). Then, the majority of arsenic in the blood is redistributed to different organs within 24 hours. The transfer process from the blood is likely to follow biexponential or triexponential course (Vahter 1983). Fast clearance half-life from the blood was estimated to be 1 to 2 hours, while the other two phases were 30 and 200 hours, respectively. Another study indicated that the clearance rate of arsenic from blood in humans was 1, 5, and 35 hours in a 3 exponential model (Tam *et al*. 1979).

Redistribution of the compounds occurs rapidly in the liver, kidney, spleen, lung, and gastrointestinal tract during the first day, incorporating arsenic into hair, nail, skin and bone within 2 to 4 weeks (Wester *et al*. 1981, Vahter 1983, USEPA 1985a, Malachowski 1990). Bone, muscle and skin are the major depots of the arsenic body burden. Furthermore, arsenic absorbed via ingestion has a shorter clearance half-life than that via inhalation (Magos 1991).

Organic arsenic that is absorbed is also distributed throughout the body, such as blood, kidney, liver, lung, muscle, and skin.

Metabolism and Excretion

Metabolism of inorganic arsenic in the human body is incompletely understood. In general, some of the dissolved arsenic (V) can be rapidly reduced to arsenic (III) in blood, while some arsenic (III) is oxidized to arsenic (V) (Vahter 1983, Malachowski 1990). Both of arsenic (III) and (V) are metabolized in the liver to methylated compounds: methylarsonic acid, dimethylarsinic acid, and trimethylarsenic compounds. Methylation results in the detoxification of inorganic compounds and then enhances the arsenic excretion.

The major excretion route for all arsenic species is by urine. A small fraction of arsenic compounds appears in the feces, bile, sweat and breast milk (Malachowski 1990). The urinary excretion rate in humans rises sharply within the first day and then declines rapidly in the following days (Tam *et al.* 1979, Farmer and Johnson 1990, Malachowski 1990). About 80 to 90% of the total inorganic arsenic excreted in urine occurs as methylated compounds (Hopenhayn-Rich *et al.* 1993). On the other hand, organic arsenic absorbed is directly eliminated in urine without metabolism (ATSDR 1989b, 1993a).

6.2.3 Bioavailability

A. Inhalation

Humans

Airborne arsenic inhaled has been found to be rapidly deposited in a respiratory tract and absorbed in the systemic circulation. There is a relationship between the inhalation of inorganic arsenic and an increase of the urinary arsenic levels (Pinto *et al.* 1977, Smith *et al.* 1977, Vahter *et al.* 1986). The extent of deposition, retention, and clearance are controlled by the size of aerosols and solubility.

Bioavailability of inorganic arsenic in human volunteers had been studied by Holland *et al.* (1959). Two volunteers who were terminal lung cancer patients inhaled sodium arsenite ($NaAsO_2$) by smoking a cigarette and being nebulized from a positive pressure machine. Using external chest counting, researchers found that 5% and 8% of arsenic contained in the cigarette, and 32% and 62% of a nebulized arsenic solution were deposited within the lung. During the first four days, 75% to 85% of the deposited arsenic was cleared from the lung, corresponding to 85% to 90% of the clearance rate after 14 days. However, the fraction of urinary and fecal excretion following 10 days dosing was 45% and 2.5%, respectively. Overall, 85% to 90% of the deposited water-soluble arsenic (III) can be bioavailable in humans.

These values are derived from the deposited dose of the compounds. Consequently, it is important to recognize the need to take into account the deposition fraction.

Animals

In animal experiments, there are differences of respiratory bioavailability among species, chemical forms and water solubility. Water soluble inorganic and organic arsenic deposited can be more rapidly absorbed (Dutkiewicz 1977, Stevens *et al.* 1977, Inamasu *et al.* 1982, Marafante and Vahter 1987).

Inamasu *et al.* (1982) investigated the degree of retention of arsenic trioxide (III) and calcium arsenate (V) in rats. One day after the intratracheal administration, only 50% of arsenic trioxide was retained in the respiratory tract. After 7 days, 1% remained in the lung. Compared to arsenic trioxide, the retention fraction of calcium arsenate at 1 and 7 days was 50%. In this study, no data were provided to indicate urinary or fecal excretion for these two compounds. Accordingly, it is difficult to determine the bioavailability factor. In another study, the high retention level of arsenic in the lung was also found in hamsters following intratracheal administration of calcium arsenate (Pershagen *et al.* 1982). However, the significantly high level of retention of calcium arsenate in the lung appears to be related to the increased incidence of lung cancer by intratracheal administration of calcium arsenate in animals.

Marafante and Vahter (1987) reported the solubility of inorganic arsenic trisulfide (As_2S_3) and lead arsenate ($Pb_3(AsO_4)_2$) *in vivo* was 80% and 10%, respectively. Following intratracheal dosing, the lung retention fractions for sodium arsenite ($NaAsO_2$), sodium arsenate (Na_3AsO_4), As_2S_3 and $Pb_3(AsO_4)_2$ were 0.06%, 0.2%, 1.3%, and 45.5%, respectively. Based on the results of total whole body retention, and urinary and fecal excretion, the bioavailability factors for these four compounds were estimated to be 80%, 71%, 75% and 47%, respectively.

Additionally, an *in vitro* solubility study revealed that 80% of arsenic trioxide dust, 16% of arsenic trisulfide dust and 23% calcium arsenate were dissolved in a saline solution at 6 days (Pershagen *et al*. 1982).

Organic arsenic was found to be readily and rapidly absorbed into the systemic circulation (Stevens *et al*. 1977). A single dose (33 μg) of dimethylarsenic acid (DMA) was intratracheally administered in rats. Only 5% of DMA remained in the lung at 15 minutes. At 24 hours, about 24% of administered DMA was retained in the body, and 60% and 8% were excreted in urine and feces, respectively. Thus, about 92% of DMA was apparently bioavailable.

B. Ingestion

Humans

Water soluble inorganic arsenic is greatly and rapidly absorbed in humans. In the study from Bettley and O'Shea (1975), seven subjects orally received 8.5 mg of Liquor arsenicalis (arsenic III). The total recovery of arsenic in feces was only 3.5% at 10 days. Accordingly, about 96% of arsenic (maximum value) was apparently absorbed into the portal blood. 52% of arsenic was eliminated in urine (minimum value).

There was a linear relationship between the daily total amount of arsenic excreted in urine and the daily absorbed amount after the equilibrium was reached (Mappes 1977, Buchet *et al*. 1981a). Under this circumstance, the amount of daily urinary excretion reflects the minimum amount of daily absorption. Buchet *et al*. (1981a) investigated the absorption of sodium arsenite ($NaAsO_2$) in four volunteers. The subjects ingested the compound at doses of 125 μg, 250 μg, 500 μg and 1,000 μg once a day for five consecutive days. After 14 days dosing, the fractions of urinary excretion were 54%, 73%, 74% and 64% for these four doses, respectively. Only 9%, 5%, 14% and 17% of parent arsenic at these four dose levels were detected in urine.

In another study, Mappes (1977) reported 62% to 72% of daily urinary excretion when one volunteer took 0.76 mg arsenic (As_2O_3) daily by oral ingestion for five days (with equilibrium). Conversely, no increase of urinary excretion took place following oral administration of 12 mg of water insoluble arsenic selenide (As_2Se_3).

Organic arsenic is bioavailable, depending on water solubility. Tam and Lacroix (1980) found only 0.21% and 0.44% of arsenic in feces from two volunteers who ingested 10 mg of fish arsenic (organic arsenic in fish). Therefore, fish arsenic was completely absorbed. In contrast, slightly water-soluble arsanilic acid was less bioavailable (Calesnick *et al.* 1966). The bioavailability factor was 26% for ingesting arsanilic acid solution, and 36% for ingesting arsenic tissue residue in chicks.

Animals

From animals studies, oral bioavailability of arsenic varies with species, chemical forms and water solubility.

Either arsenic (III) or arsenic (V) was completely absorbed in mice (Vahter and Norin 1980). Sodium arsenite ($NaAsO_2$) (III) and sodium arsenate (Na_2HAsO_4) (V) were administered to mice by gavage and subcutaneous at dose of 0.01 mg and 0.1 mg. Two days latter, the fractions of fecal excretion by oral and subcutaneous injection were 8% and 6% for arsenic (V), and 7% and 4% for arsenic (III), respectively. The fraction of fecal excretion by subcutaneous injection represented biliary excretion. With a correction, the bioavailability factors were 98% and 97% for these two compounds.

The extent of absorption of arsenic trioxide in rats and rabbits was similar, being 31% at 4 days and 37% at 7 days (fecal excretion data), respectively (Ariyoshi and Ikeada 1974). But the absorption of arsenic trioxide in monkeys was extremely high during 14 days following oral administration of the compound (1 μg/kg) (Charbonneau *et al.* 1978). The bioavailability factors ranged from 73% (urinary excretion data) to 98% (fecal excretion data).

Arsenic pentoxide (As_2O_5) has a relatively low bioavailability in hamsters (Charbonneau *et al.* 1980). The compound in solution was given to hamsters by gavage or intravenously at doses of 0.01 μg arsenic. During 24 hours, 70% of the compound by gavage and 6% by intravenous were eliminated in feces. Consequently, about 34% of the compound was absorbed. However, arsenic pentoxide was found to be highly absorbed in dogs (95%) after the first day administration (Hollins *et al.* 1979).

Bioavailability of inorganic arsenic compounds by ingestion greatly depends on the water solubility. In the experiment from Marafante and Vahter (1987), the bioavailability factors (urinary excretion data plus whole body retention) in hamsters were 43% for $NaAsO_2$ (water-soluble), 81% for

Na_3AsO_4 (water-soluble), 13% for As_2S, (slightly water-soluble), and 24% for $Pb(AsO_4)_2$ (slightly water-soluble). Compared to the study in mice (Vahter and Norin 1980), the degree of absorption of $NaAsO_2$ was lower in hamsters.

The bioavailability factor for an organic arsenic, methylarsine sulfide (MAS), was reported to be 64% in rabbits (Ariyoshi and Ikeda 1974). About 66% of DMA was bioavailable in rats (Stevens *et al.* 1977). About 90% of fish arsenic in monkeys was absorbed (Charbonneau *et al.* 1978).

Solubility Study

One study reported that about 60% of arsenic bound to soil was dissolved in a pH 2 aqueous solution at 6 hours (Murphy and Toole 1989).

C. Dermal

A few studies have shown that the absorption of inorganic arsenic (e.g. arsenic acid and arsenic trichloride) through the skin occurred in humans and animals (WHO 1981, Vahter 1983). To date, quantitative data on the dermal bioavailability is limited. Dutkiewicz (1977) reported the dermal permeability of 2.7×10^{-6} cm/h in rats after applying 0.1M sodium arsenate solution to the rat skin.

D. Summary

Overall, either inorganic or organic arsenic compounds are bioavailable in humans and animals by inhalation, ingestion and dermal (Table 6-5, 6-6, and 6-7). Quantitative data are available for inhalation and ingestion exposure routes (Figure 6-2). Water soluble compounds have a higher absorption fraction than water insoluble compounds.

6.2.4 Uncertainties

At the contaminated sites, arsenic pentoxide (water-soluble) have contaminated the surface soil. Airborne arsenic aerosols may be transported to residential areas by physical processes. Airborne water soluble arsenic is a potential health hazard for residents.

Water soluble arsenic tends to be highly bioavailable in the respiratory tract in humans. The reported bioavailability factors are derived from experiment with terminal lung cancer patients. These values may differ from normal healthy persons. On the other hand, the inhalable fraction and arsenic particle size in the air as well as deposition fraction in the respiratory tract are important considerations in the assessment of respiratory bioavailability. However, the inhalable fraction of arsenic aerosols distributed at the sites and arsenic particle size are not well documented. The deposition fraction of arsenic inhaled in the lung has been estimated from smoking or other artificial exposure. This rarely reflects the value of a normal inhalation pattern. The net absorption of inhaled arsenic is extremely dependent on the deposition fraction of the arsenic-bearing particles.

Arsenic pentoxide in the soil and lagoons at the contaminated sites may leach to groundwater or be removed to surface water by runoff and fallout. Quantitative data on water solubility for soil-bound arsenic pentoxide under natural or physiological conditions are lacking.

One major concern is whether potable groundwater near the sites has been contaminated because of the apparent association between an increased incidence of skin cancer, and perhaps some internal tumors, resulting from exposure to inorganic arsenic in drinking water. Chromated copper arsenate is found to have a relatively low solubility at physiological pH but no quantitative data are available (Vahter 1988).

Currently, there are no good animal models for the carcinogenicity of inorganic arsenic and no confirmed active arsenic species responsible reaching the target tissues. Some studies indicated that the detoxification process was likely to be saturated at about 200-250 µg As/day ingested for adults (Buchet *et al.* 1981b, USEPA 1990c, North 1992). Whether the methylated species can act as a carcinogens is not currently clear. Hence, non-methylated arsenic is generally assumed to be a probable active species when the methylation process become saturated, namely, at a methylation threshold of 250 µg/day.

The USEPA Science Advisory Board (SAB, 1989) recommended that the risk assessment for arsenic in drinking water be based on the delivered dose of non-detoxified arsenic to active sites. Recent bioavailability factors from either humans or animals result from the measurement of urinary or fecal excretion of arsenic. The results from fecal excretion represent the amount of inorganic arsenic prior to metabolism in the liver. However, the hepatic first-pass effect will dramatically influence the degree of absorption of inorganic arsenic in the systemic circulation. Thus, these values for inorganic arsenic are used as the upper bound of bioavailability factors. Because organic arsenic compounds are rapidly excreted in urine without presystemic metabolism, the values for organic arsenic compounds from fecal excretion appear to be close to their actual bioavailability factors. The values from urinary excretion are the lower bound of bioavailability factor. However, some studies only provide the total arsenic amount in urine without distinguishing parent compounds from their metabolites. Hopenhayn-Rich *et al.* (1993) reviewed the relationship between the exposure levels of inorganic arsenic and the non-methylated arsenic levels in urine from humans. Their results revealed that the levels of non-methylated arsenic in urine were fairly constant, approximately 20%, regardless of any increase of absorbed dose. These results raise questions about methylation threshold hypotheses.

In brief, uncertainties for health risk assessment for inorganic arsenic involve a lack of knowledge of toxicokinetics and mode of action.

6.2.5 Conclusions

Humans are routinely exposed to arsenic at low levels by inhalation, ingestion and dermal exposure. Inhalation of high levels of airborne arsenic primarily takes place in occupational settings or possibly at residential areas near contaminated industrial locations. Ingestion of inorganic arsenic through consumption of contaminated drinking water would pose a potential hazard for general populations. Exposure to arsenic via ingestion of soil and foods is less significant because of the generally low content of arsenic in these media. Dermal exposure route is considered to be minor for the general population.

Either inorganic or organic arsenic can be bioavailable via three exposure routes. Quantitative data in humans and animals are available for inhalation and ingestion routes. Water-soluble inorganic arsenic is extensively and rapidly absorbed in the respiratory tract. Respiratory bioavailability for water soluble arsenic deposited within the human lung may be as high as 85% to 90%. However, the net absorption in the respiratory tract strongly depends on the deposition fraction. The deposition fraction of water-soluble inorganic arsenic is about 40% in humans. USEPA assumes a values of about 30% absorption for airborne arsenic (USEPA 1984b). In small rodent animals, the respiratory bioavailability factors for slightly water soluble inorganic arsenic range from 47% to 75%.

The oral bioavailability factors for water soluble inorganic and organic arsenic range from 52% to 100%. Currently, these upper bound values (90% to 100%) are recommended to be used as the oral bioavailability factor for arsenic risk assessment and toxicity studies by WHO. For slightly water soluble organic arsenic, the oral bioavailability factors range from 26% to 36% in humans. Only 13% to 24% of slightly water soluble inorganic arsenic can be absorbed in the gastrointestinal tract in small rodent animals. For ingestion of arsenic contaminated soil, the dissolution rate in the gastrointestinal tract plays a key role in the determination of oral bioavailability. About 60% of arsenic may leach from arsenic-bearing soil matrix at acidic aqueous solution (pH 2).

The degree of bioavailability is strongly governed by water solubility of arsenic. Arsenic pentoxide is a water soluble compound. But chromated copper arsenate or soil-bound arsenic may have a relatively low water solubility in an acidic environment. The bioavailability for this type of arsenic is expected to be lower than that for a single arsenic pentoxide.

6.2.6 Recommendations for Further Evaluation and Research

For respiratory bioavailability studies, it is essential to determine the inhalable fraction of airborne arsenic aerosols and arsenic aerosol size distribution where inhalation exposure occurs at contaminated sites, so that the deposition fraction of arsenic inhaled within the respiratory tract can be estimated.

Because exposure to some inorganic arsenic via ingestion by humans leads to skin cancer and other, internal tumors as well as systemic toxicity, the internal dose and delivered dose through the blood and other target tissues are critical for arsenic risk assessment. Accordingly, it is necessary to identify active arsenic species, to investigate the mechanism of arsenic metabolism, to determine the delivered dose through the blood and other target tissues, and to establish quantitative relationships between the dose of active species and arsenic toxicity or carcinogenicity.

Quantitative solubility studies for soil-bound arsenic at physiological conditions and bioavailability studies for chromated copper arsenate are needed.

Absorption by dermal exposure is poorly understood. Further research in this field is needed.

Table 6-5 Respiratory Bioavailability of Arsenic in Humans and Animals

Compound	Species	Administered method	Arsenic dosage	Experimental method	Exposure duration	As Retention in the lung	As Recovery			Bioavailability factor	Ref.
							Whole body	Urine	Feces		
Inorganic											
(1)NaAsO$_2$	Human	Smoking Nebulizing	200-300 μc	External counting; Urinary and fecal excretion	4 d 10 d 14 d	15-25% 10-15%		45%	2.5%	75-85%(3) 85-90%(3)	a
(1)NaAsO$_2$	Hamster	Intra-tracheal	100 μg (2 mg/kg)	Whole-body counting; Urinary and fecal excretion	3 d	0.06%	25%	55%	15%	80%	b
(1)Na$_3$AsO$_4$						0.02%	15%	56%	16%	71%	
(2)As$_3$S$_3$						1%	15%	61%	15%	75%	
(2)Pb$_3$(AsO$_4$)$_2$						46%	60%	33%	6%	47%	
Organic											
(1)Dimethyl-arsinic acid	Rat	Intra-tracheal	33 μg	Whole-body counting; Urinary and fecal excretion	15 min 24 hr	5%	24%	60%	8%	92%	c

(1): Water soluble; (2): Slightly water soluble; (3) Data from the deposited compounds within the respiratory tract
Adapted from a: Holland *et al*. 1959; b: Marafante and Vahter 1987; c: Stevens *et al*, 1977

Table 6-6 Oral Bioavailability of Arsenic in Humans

Compound	Number of Subject	Arsenic carrier	Arsenic dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Inorganic							
(4)Liq. Arsenicals [As (III)]	11	Water	8.5 mg	Urinary and fecal excretion	10 d	97%(1) 52%(2)	a
(4)$NaAsO_2$	4	Water	125 μg x 5 250 μg x 5 500 μg x 5 1,000 μg x 5	Urinary excretion	14 d	54%(2) (9%)(3) 73%(2) (5%)(3) 74%(2) (14%)(3) 64%(2) (17%)(3)	b
(4)As_2O_3	1	Water	0.76 mg x 5	Urinary excretion	5 d	69-72%	c
(6)As_2Se_3	1	Powder	12 mg			0%	
Organic							
(4)Fish arsenic	2	Meal	10 mg	Urinary and fecal excretion	-	99.7%	d
(5)Arsanilic Acid	4	Water Chick (fed As)	1.6-3.0 mg 160-210 g of pate	Urinary and fecal excretion	6 d	26% 36%	e

(1): the values from fecal excretion data that are considered as the upper bound of bioavailability factor; (2): the values from urinary excretion data (parent compound and its metabolites) that are considered as the lower bound of bioavailability factor; (3): the values are the fraction of the parent compound in urine; (4): Water soluble; (5): Slightly water soluble; (6): Water insoluble

Adapted from a: Bettley and O'Shea 1975; b: Buchet *et al*. 1981a; c: Mappes 1977; d: Tam and Lacroix 1980; e: Calesnick *et al*. 1966

Table 6-7 Oral Bioavailability of Arsenic in Animals

Compound	Species	Administration methods	Arsenic carrier	Arsenic dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Inorganic (4)$NaAsO_2$ (4)Na_2HAsO_4	Mouse	Gavage	Water	0.4-4 mg/kg	Urinary and fecal excretion; Whole body counting	2 d	98%(1) 97%(1)	a
(4)As_2O_3	Rat Rabbit	Feed	Diet	29 mg/kg	Urinary and fecal excretion	4 d 7 d	31%(1) 37%(1)	b
(4)As_2O_5	Hamster	Gavage	Water	0.01 μg (0.1 μg/kg)	Urinary and fecal excretion; Whole body counting	1 d	34%(1)	c
(4)$NaAsO_2$ (4)Na_3AsO_4 (5)As_2S_3 (5)$Pb_3(AsO_4)_2$	Hamster	Oral	Water	2 mg/kg	Urinary and fecal excretion; Whole body counting	3 d	51%(1)(43%)(3) 88%(1)(81%)(3) 17%(1)(13%)(3) 31%(1)(24%)(3)	d
(4)As_2O_5	Dog	Oral	Gelatin capsule	0.3 μg (0.02-0.03 μg/kg)	Urinary and fecal excretion; Whole body counting	1 d	95%(1)	e

Adapted from a: Vahter and Norin 1980; b: Ariyoshi and Ikeda 1974; c: Charbonneau *et al*. 1980; d: Marafante and Vahter 1987; e: Hollins *et al*. 1979;

(Continued)

Compound	Species	Administration methods	Arsenic carrier	Arsenic dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
(4)As_2O_3	Monkey	Gavage	Meal	1 µg/kg	Urinary and fecal excretion	14 d	98%(1) 73%(2)	f
Organic								
(4)Methylarsine sulfide	Rabbit	Feed	Diet	36 mg/kg	Urinary and fecal excretion	7 d	64%	b
(4)Fish arsenic	Monkey	Feed	Meal	1 µg/kg	Urinary and fecal excretion	14 d	90%	f
(4)Dimethyl-arsinic acid	Rat	Oral	-	0.1µg/kg	Urinary and fecal excretion whole-body retention	24 hr	66%	g

(1): the values from fecal excretion data that are considered as the upper bound of bioavailability factor; (2): the values from urinary excretion data (parent compound and its metabolites) that are considered as the lower bound of bioavailability factor; (3): the values are the fraction of the urinary excretion data plus whole body retention; (4): Water soluble; (5): Slightly water soluble
Adapted from f: Charbonneau *et al*. 1978; g: Stevens *et al*. 1977

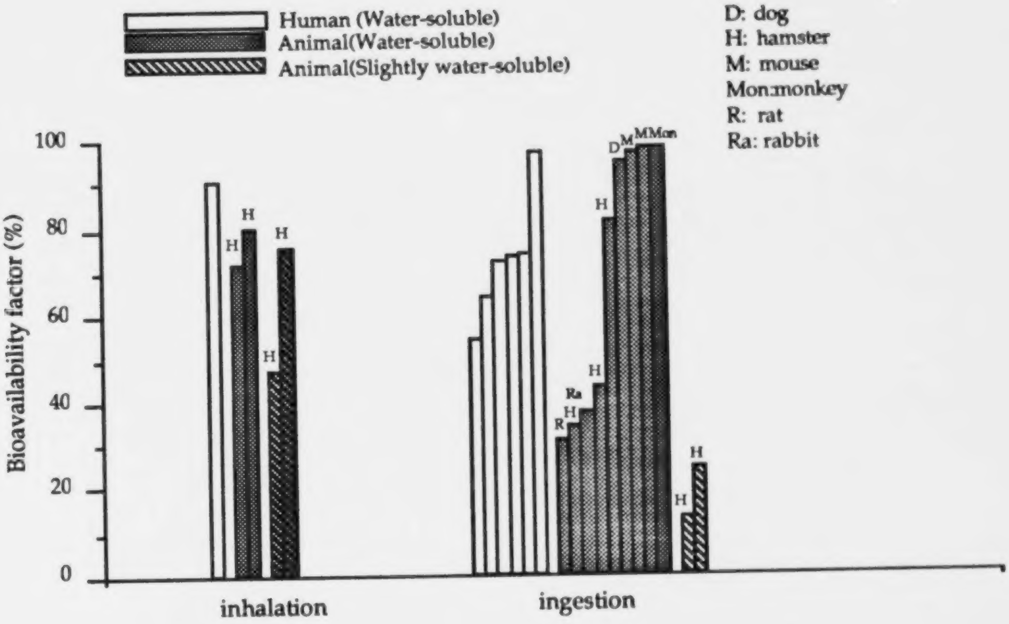

Figure 6-2 Bioavailability factors of inorganic arsenic in humans and animals by three exposure routes [Adapted from sources in Table 6-5: a,b (inhalation); Table 6-6: a,b,c (ingestion); and Table 6-7: a,b,c,d,e,f(ingestion)].

6.3 Copper

Copper is widely distributed in the environment, with a variety of concentrations ranging from levels that may be deficient to those that are toxic for humans. There exist many oxidation states from 0 to +3 , but the free copper (II) ions have the main biological significance (Seiler and Sigel 1988, ATSDR 1990b).

The information about the form of copper in the air is limited. Copper may exist as oxides, sulfate or carbonate (ATSDR 1990b). In water, copper occurs as Cu^{2+}, $Cu(HCO_3)^+$, and $Cu(OH)_2$. Copper (II) also forms a number of stable complexes with inorganic or organic ligands or is bound to the particulate matter in water (Stiff 1971). Large amounts of copper from various sources are released to soils and sediments (USEPA 1980a). The forms of copper in these media depend on the soil or sediment conditions, such as pH, Eh, types of soils and the amount of organic matter in soil and sediment (USEPA 1979a, 1980a). Copper is essential for the normal growth and development of plants. In natural conditions, copper rarely accumulates to high levels in plants (ATSDR 1990b). The concentrations of plant copper may elevate with an increase in soil copper levels. As a trace element, humans take copper from their daily diet. For example, adults require approximately 1.5 to 3 mg/d for normal health (NAS 1989).

As an essential nutrient, copper plays an important role in human metabolism (Harris 1991). Because there is limited evidence of severe toxic effects in humans following exposure to copper, copper is usually not considered to be a particularly hazardous industrial substance (Carson *et al.* 1986, ATSDR 1990b, ACGIH 1991).

Humans take a certain amount of copper from their diet and drinking water to satisfy their daily metabolic requirement. Absorption of copper in the gastrointestinal tract is a homeostatic process. Hence, inhalation and dermal exposure routes may be of great concern. Currently, quantitative estimates of copper bioavailability by inhalation and dermal contact is limited.

6.3.1 Exposure Routes and Receptors

Humans can be exposed to copper by inhalation, ingestion and dermal contact because copper is widely distributed in various environmental media. In principle, only the free copper (II) ion or weakly complexed copper, as an available form, is a potential health hazard for humans (ATSDR 1990b). In effect, the majority of copper in the environment is tightly bound to organic or mineral matter, with the bound forms being less toxic for humans.

In the occupational environment, workers may be exposed to high levels of copper fumes and dusts via inhalation (ACGIH 1991). Dermal exposure may occur for workers who deal with soluble cupric salts.

Although people ingest a large amount of copper daily from various foods, absorption of copper in the gastrointestinal tract tends to be restricted to levels that satisfy metabolic requirements. The average daily intake of dietary copper is less than 2 mg (ATSDR 1990b). Accordingly, ingestion of copper from foods is unlikely to be a significant exposure route for humans.

An excess amount of the free copper (II) ions may be present in drinking water when copper pipes are used with corrosive water. In such cases, ingestion of drinking water may become an important exposure route for the general population.

Copper sulfate may be used to control blooms of algae. In such cases, humans may be exposed to high levels of free form of copper dermally during bathing or swimming. Additionally, residents living near copper smelters may be exposed to high levels of copper-containing soil or dust.

Exposure to copper via inhalation or dermal contact may be substantial in very special circumstances for the general or occupational population. Quantitative information about bioavailability by these two exposure routes is limited.

6.3.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.3.3 on Bioavailability.

Distribution

Once absorbed, the distribution of dietary copper takes place in two stages (Goode *et al.* 1989). First, copper is bound to the albumin and amino acids in the portal blood. Upon arrival at the liver, the copper released is transported to the hepatocytes where it is incorporated into various copper proteins, such as ceruloplasmin (Marceau *et al.* 1970). Then, ceruloplasmin as well as a small fraction of albumin-/amino acid-copper is released into the systemic circulation and taken up by extrahepatic organs. Alternatively, the copper complexes are excreted via the biliary tract if a large amount of copper is present in the liver. Consequently copper levels in the liver usually remain relatively constant making the liver a major homeostatic organ for copper (Harris 1991).

The highest concentrations of copper are found in the liver, brain, heart and kidney (Harris 1991). The lung, intestine and spleen, bone and muscle contain relatively low copper levels. The total body burden of copper is typically about 80 mg to 120 mg. Approximately 10% of the total copper burden is enriched in the liver (Seiler and Sigel 1988).

Metabolism and Excretion

There is no free form of copper in the human body. Absorbed copper is soon complexed with a variety of proteins, amino acids and peptides. Copper, as a component of the molecular structure in these proteins, acts as a co-factor of some enzymes.

In the cytosol of hepatocytes, 80% of copper is incorporated to hepatocuprein, copper-chelatin, methallothionein, and other specific copper proteins (NAS 1977b). Of all the important copper proteins, ceruloplasmin, a blue plasma glycoprotein, is considered to be a main transfer agent for copper because it accounts for 70% to 90% of copper in plasma (Harris 1991).

Based on excretion studies via oral and intravenous exposure, copper is observed to be primarily eliminated via bile in fecal excretion. Biliary copper is seldom reabsorbed in the intestine because of being readily bound to proteins. The rest of fecal copper results from the salivary, gastric, pancreatic, and jejunal secretions, the intestinal mucosae, as well as nonabsorbed dietary copper (NAS 1977b). Urinary excretion of copper plays a minor role, being only 2% to 4% of the total excretion of copper (Triebig and Schaller 1984).

6.3.3 Bioavailability

A. Inhalation

Absorption of copper may result from the inhalation of copper fumes and dusts in an occupational environment (Triebig and Schaller 1984). But there are no quantitative bioavailability data via inhalation documented for humans or animals. One study indicated that the retention of copper within the lung declined sharply at 24 hours after the intratracheal administration of Whetlerite dust in rats (Salem and Katz 1989).

B. Ingestion

As a trace element, the oral bioavailability of dietary copper has been well studied in humans and animals. In principle, the absorption and excretion of dietary copper are regulated in accordance with the daily requirements for copper in humans and animals. Therefore, the toxicity and deficiency of copper rarely occur by the oral exposure to copper unless individuals have copper-related diseases, such as Wilson's disease or Menkes' disease.

The main absorption sites for copper are located in the stomach and duodenum in humans (Bearn and Kunkel 1955, Weber *et al.* 1969). Based on the animal experiments, there are two mechanisms involved in the absorption of copper. One includes a specific carrier for copper ions in the epithelial cells of the gastrointestinal tract (Harris 1991). Other metal ions, such as cadmium, iron, and zinc, may compete for the enzymatic carrier (King *et al.* 1978, Kies and Harms 1989, Greger 1992). The second mechanism is associated with the absorption of an amino acid-copper complex which is formed by the binding of methallothionein in the intestinal mucosa (Mason 1979, Harris 1991).

Various factors can influence the oral bioavailability of copper (Johnson 1989, Johnson *et al.* 1992). Besides the metal ions, age, gender, pregnancy, diseases, types of food, and ascorbic acid can be attributed to variable absorption of copper.

Well-documented quantitative human bioavailability data for ingestion have been reported in the literature (Cartwright and Wintrobe 1964, Sternlieb 1967, Weber *et al.* 1969, Strickland *et al.* 1972, Dekaban *et al.* 1974, King *et al.* 1978, Nageswara Rao 1980, Turnlund *et al.* 1982, 1983, 1985, 1988a, 1988b, 1989, Jacob *et al.* 1987, Johnson *et al.* 1988, 1992, Turnlund 1989). In these studies, volunteers took dietary copper and labeled copper solutions by both oral and intravenous routes at various levels and durations. Quantitative results were usually obtained by using a whole-body counting technique or fecal excretion monitoring. In effect, copper absorption might be underestimated by using the fecal excretion method. Fecal copper represents the portion of nonabsorbed copper and endogenous losses of copper. Absorbed copper excretion in feces is estimated to be approximately 9% to 16% (Mason 1979, Turnlund 1989).

From a variety of studies in humans, the average values of absorption of dietary copper range from 14% to 71% (Table 6-8 and Figure 6-3). The variation of bioavailability is strongly dependent on the daily intake of dietary copper. (Figure 6-5). Within daily required levels (around 1.5 to 3 mg/d), about 20% to 40% of dietary copper was absorbed. With an increase of administered copper to 7.5 mg/d, the oral bioavailability declined to 14%, while the absorption fraction increased to 50% to 70% at a deficient daily copper intake level (around 1 mg/d).

C. Dermal

Absorption of copper via dermal exposure has been reported (Bentur *et al.* 1988 and Hostynek *et al.* 1993). Little information on quantitative dermal absorption is available for humans and animals. A few *in vivo* and *in vitro* studies revealed that copper combined with various carriers could penetrate the skin (Walker and Reeves 1977, Beveridge *et al.* 1984).

6.3.4 Uncertainties

Water-soluble copper (II) could be released to surface soils and tightly bound to the organic matter at specific contaminated sites. Residents near the waste sites may be exposed to soil-bound copper in airborne dust.

Indeed, the copper forms, concentration and particle size distribution in the air are not known at the contaminated sites. However, based on data from other studies, most airborne copper could present in the complexes with sulfur.

There have been a few reports of adverse health effects by inhalation of fumes and dusts in occupational settings. Toxicity by inhalation for the general population is poorly understood. Furthermore, quantitative data on the bioavailability via inhalation in both humans and animals are very limited.

Although copper may contaminate surface and groundwaters, and surface soils, the occurence of toxic effects via ingestion is dependent on the amount of free copper (II) ions or weakly bound complexes in these media. The ratio of free copper (II) ions to the complexes at the specific contaminated sites is not known. In bioavailability studies, differences in absorption for different copper (II) compounds in the gastrointestinal tract is also not well characterized.

Toxicity and absorption of copper by dermal exposure has received little attention. Thus, many uncertainties are involved in the assessment of dermal exposure.

6.3.5 Conclusions

Although the ingestion of copper from foods and drinking water is a major exposure pathway for the general population, toxicity rarely occurs in healthy humans because of homeostasis. A potential health hazard posed by oral exposure is minor, with exception of exposure to excessive free copper (II) in drinking water. Inhalation and dermal exposure may be significant for occupational settings. Residents living near industrial areas may be exposed to high levels of airborne copper.

Humans require a certain amount of copper for daily metabolic needs. This is usually satisfied by ingestion of dietary copper (about 1.5 to 3 mg/d). Absorption and excretion of dietary copper are regulated. The mean values of bioavailability factor range widely from 14% to 71% in humans, depending on the daily intake of dietary copper. Quantitative bioavailability data for inhalation and dermal exposure are not currently available.

6.3.6 Recommendations for Further Evaluation and Research

Compared to chromium and arsenic, copper posses a relatively low health risk for humans. The health risk for ingestion of copper may be negligible. In contrast, exposure by inhalation and dermal contact may be a greater concern. For the evaluation of respiratory and dermal bioavailability, the following data are needed: the forms of copper in the environmental media, particle size distribution, inhalable fraction, the rate of deposition-retention-clearance within the respiratory tract, and the permeability of copper on the skin.

Table 6-8 Oral Bioavailability of Copper in Humans

Copper Compound	Number of Subjects	Administered method	Copper dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Dietary copper	235	Oral	2-5 mg	Body fluid measurement	-	32%	a
^{64}Cu	49	Oral/i.v.	2mg	Serum level measurement	2 d	40%	b
Copper acetate	7	Oral/i.v.	Single: 300 µg	Whole-body counting	4 d	60% (15-97%)	c
Copper acetate	4	Oral/i.v.	0.4-4.5 mg	Whole-body counting	20 d	56% (40-70%)	d
Cupric chloride	1	Oral/i.v.	-	Whole-body counting	8 d	52%	e
Formula copper	8	Oral	3.2 mg/d	Fecal excretion	10-12 d	57%	f
Dietary copper	6	Oral	2.4 mg/d	Fecal excretion	11 d	18% (7-37%)	g
Dietary copper	7	Oral	1.25 mg/d	Fecal excretion	5.5 mo	50-65%	h

Adapted from a: Cartwright and Wintrobe 1964; b: Sternlieb 1967; c: Weber *et al.* 1969; d: Strickland *et al.* 1972; e: Dekaban *et al*, 1974
f: King *et al.*, 1978; g: Nageswara Rao, 1980; h: Johnson *et al.*, 1988

(Continued)

Compound	Number of Subject	Administered method	Dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Formula copper or dietary copper or both forms	4-11	Oral	0.8 mg/d	Fecal excretion	42-98 d	56%	i
			1.4 mg/d			41%	
			2.1 mg/d			34.4%	
			2.3 mg/d			33.5%	
			2.5 mg/d			33.8%	
			2.7 mg/d			27.5%	
			3.3 mg/d			25.8%	
			7.5 mg/d			14%	
Dietary copper	127	Oral	1.3 mg/d in men	Whole-body counting	21 d	64% (20-59y)	j
			1.1 mg/d in women			71% (20-59y)	

Adapted from i: Tuenlund 1989 (Data from seven studies); j: Johnson *et al*. 1992

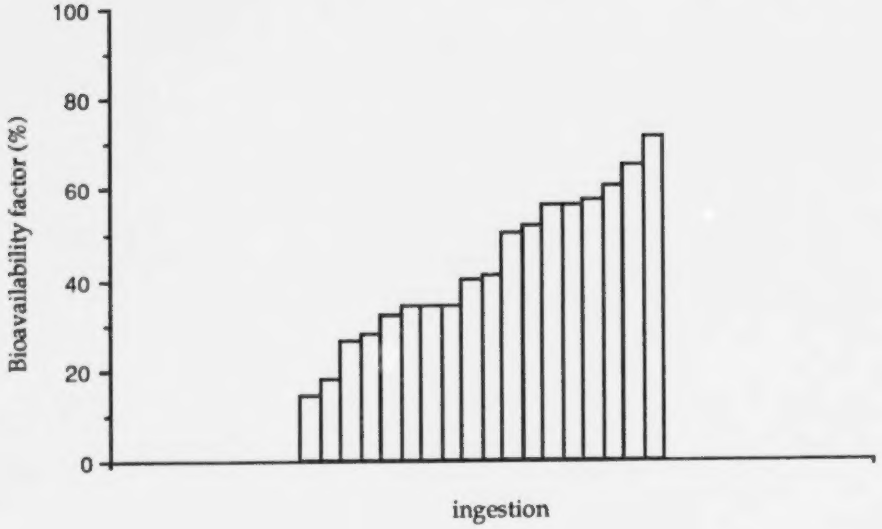

Figure 6-3 Oral bioavailability factors of copper in humans [Adapted from source: Table 6-8 a-j]

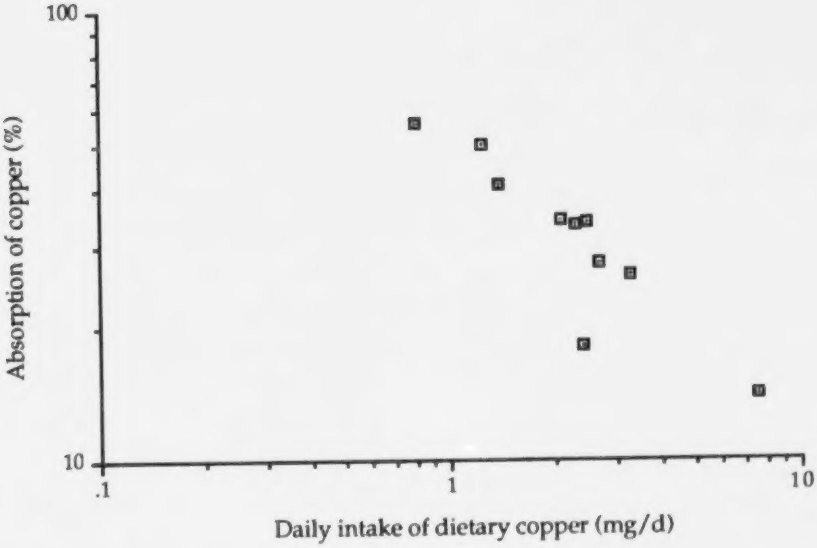

Figure 6-4 A plot of bioavailability factor vs. daily intake of dietary copper (Adapted from Nageswara Rao 1980, Johnson *et al.* 1988, Tuerlund 1989).

6.4 Benzene

Benzene is a highly volatile, relatively nonpolar aromatic hydrocarbon that is widespread in the environment (Sittig 1991). Benzene is slightly water soluble and highly miscible with organic solvents, such as alcohol, ether, acetone and chloroform (IARC 1982a). This hydrocarbon has an octanol/water partition coefficient (log k_{ow}) of 2.13 and soil adsorption coefficient (log k_{oc}) of 1.8 to 1.9 (ATSDR 1991a, 1993b, Lide 1993).

Once released to the environment, generally, over 99% of benzene will partition into the air as a vapor. Based on physicochemical properties, there is exchange between air and water/soil (HWC 1979). Benzene in air is readily degraded by reaction with hydroxy radicals (USEPA 1985a, ATSDR 1991a, 1993b). A very small fraction of benzene will partition into surface water, soil and sediment (IARC 1982a, Hattemer-Frey *et al.* 1990, Howard 1990a). The biodegradation of benzene in surface water and soil may occur, depending on the presence of an active microbial population, dissolved oxygen and nutrients. Plants can be contaminated by benzene through deposition, root uptake, and air-to-leaf transfer (Bedleman 1988, Hattemer-Frey *et al.* 1990). Some foods naturally contain benzene (USEPA 1978). Quantitative data for benzene have been reported in several foods: eggs, Jamaican rum, nuts, dairy products, fish, irradiated beef and heat-treated canned beef (USEPA 1980b, IARC 1988).

Benzene may produce a series of toxic effects in various human organs. In particular, benzene induces hematological disorders and is also a recognized human carcinogen by inhalation (Ellenhorn and Barceloux 1988, IARC 1982a).

Bioavailability of benzene is high through inhalation and ingestion exposure, whereas it is very low by dermal exposure in humans and animals because of its high volatility.

6.4.1 Exposure Routes and Receptors

Humans are primarily exposed to benzene by inhalation of vapor and dermal contact with vapor and liquid. Ingestion of benzene through water, food and soil, or dermal contact with soil-associated benzene are potential routes for the general population.

Occupational exposure to benzene frequently occurs via inhalation because of elevated levels in the workplace (OSHA 1987, 1989). Workers may be also exposed to benzene through dermal contact (ATSDR 1991a, 1993b).

The primary exposure route for the general population is inhalation of benzene vapor indoors and outdoors (Brunnemann *et al.* 1989, Hajimiragha *et al.* 1989, Wallace 1986, 1989). Cigarette smoking is the most significant indoor source. Individual exposures to benzene indoors are about two times higher than those outdoors, accounting for 50% of the total population exposure (Wallace 1986, 1989). Sources of atmospheric benzene outdoors come from emissions of automobile exhaust, waste industrial sites, and manufacturing chemical sites. However, benzene released to outdoor air only contributes about 20% of the total population exposure (Wallace 1986, 1989).

Because less than one percent of benzene in the environment partitions into water, soil and biota, the potential hazard through ingestion and dermal exposure is minor for the general population (Hattemer-Frey *et al.* 1990). Although benzene occasionally appears in drinking water and is detected in a few foods, the total population exposure is insignificant in comparison with the exposure by inhalation (Wallace 1986, 1989). Dermal contact with soil-associated benzene will also be negligible. Only direct dermal exposure to liquid solvents containing benzene is likely to pose a serious exposure risk.

6.4.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.4.3 on Bioavailability.

Distribution

Once absorbed following inhalation exposure, benzene is soon distributed to the blood, brain, liver, kidney, stomach and fat in humans (Tauber, 1970; and Winek and Collom, 1971). Animal experiments have shown benzene and its metabolites, through ingestion and dermal exposure, could reach the blood, bone marrow, liver, kidney, lung, brain, untreated skin and fat (ATSDR 1991a, 1993b). The parent compound and its metabolites are stored in the adipose tissue and bone marrow.

Metabolism

The toxicity of benzene has been attributed to its metabolites. Metabolism mainly takes place in the liver. Then, benzene metabolites may be transported to the bone marrow via the blood stream for further metabolism or direct toxic action on blood cells.

There are several pathways involving either increased toxicity or detoxification of benzene (IARC 1982a, Sabourin *et al.* 1988, 1989; Henderson *et al.* 1989, Eastmond *et al.* 1987, Kalf 1987, Travis *et al.* 1990, ATSDR 1991a, 1993b, Andrews and Snyder 1991, Cox 1991). Benzene is initially metabolized to benzene oxide by cytochrome P-450 dependent mixed-function oxidase enzymes in the liver. In the detoxification processes, part of benzene oxide is conjugated to glutathione to form phenyl-mercapturic acid, which is excreted from the body. Alternatively, benzene oxide can be rearranged to form phenol, some of which become conjugated to glucuronide and sulfate to form water-soluble complexes that are readily excreted in the urine.

On the other hand, benzene can be bioactivated via other pathways. Benzene oxide may be converted to dihydrodiol by epoxide hydrolase, which is oxidized to catechol by dehydrogenases. Small proportions of catechol may result from the hydroxylation of phenol. Hydroquinone, the most toxic metabolite, is also generated by the hydroxylation of phenol. As a minor metabolite, muconaldehyde comes from breaking the benzene ring. Phenol, catechol and hydroquinone are the principal hepatic metabolites of benzene.

Phenol, catechol and hydroquinone can reach the bone marrow. In the bone marrow, phenol is rapidly oxidized to hydroquinone by the marrow peroxidases, which leads to the formation of benzoquinone by further oxidation. Catechol may convert to trans-trans-muconaldehyde by dioxygenases. These products can interact with cellular DNA, nuclear protein and cell surface membrane protein.

Overall, phenol, catechol and hydroquinone, so-called transport forms, play a key role in determining the metabolism of benzene in the bone marrow. The toxic hepatic metabolites may directly act at the target sites. Alternatively, they are activated in the bone marrow by other enzyme systems.

Excretion

Elimination of benzene and its metabolites depends on the exposure routes. By inhalation, about 12% to 17% of benzene is exhaled unchanged (ATSDR 1991a, 1993b). Through other exposure routes, small fractions of benzene absorbed may be eliminated via the expired air. The majority of the parent compound and its metabolites are excreted via urine, and, to lesser extent, via feces. Conjugated phenol is a primary product in urine (Sherwood 1988).

6.4.3 Bioavailability

A. Inhalation

Humans

Benzene vapor is rapidly absorbed into the human body after inhalation. Various factors can influence the respiratory bioavailability of benzene. Because of a slight water solubility with the blood/gas partition coefficient of (log) 7.8 (Sato and Nakajima 1979), inhaled benzene is incompletely absorbed into the systemic circulation.

In human studies, volunteers inhaled benzene vapor by whole-body or oral-only exposure. The exposure concentration of the vapor in the air ranged from about 47 to 110 ppmv, with 1 to 4 hours of exposure duration. The extent of absorption was determined by measuring exhalation of benzene.

Results from those studies indicated the bioavailability factors ranging from 47% to 60% following post-exposure of 2 to 4 hours (Srbova *et al.* 1950, Hunter 1968, Nomiyama *et al.* 1974). But a large amount of benzene vapor (70 to 80%) was absorbed during 5 minutes of post-exposure, and then declined sharply (Srbova *et al.* 1950). The concentration of benzene in the blood reached a steady state within 30 minutes.

Animals

From animal experiments (Schrenk *et al.* 1941, Snyder *et al.* 1981, Sabourin *et al.* 1987), the absorption of benzene vapor was variable with the exposure concentration, exposure duration, and species. The degree of absorption for benzene declined through progressive exposure (Snyder *et al.* 1981, Sabourin *et al.* 1987).

B. Ingestion

There are a few studies indicating that benzene can be absorbed in the gastrointestinal tract in humans (ATSDR 1991a, 1993b). Little information on quantitative bioavailability by ingestion has been published.

Benzene can be extensively absorbed from the gastrointestinal tract in animals. In rabbits, 84% to 89% of liquid benzene was absorbed following gavage administration at a single dose of 0.4 g/kg (Parke and Williams 1953). In another study, the bioavailability factor was 97% for rats and mice after oral exposure to benzene at a dose of 0.5 to 150 mg/kg b.w. (Sabourin *et al.* 1987). When benzene was adsorbed to different kinds of soils (sandy and clay soil), bioavailability was altered (Turkall *et al.* 1988, Abdel-Rahman and Turkall 1989, Travis and Bowers 1990). After oral administration of pure benzene (150 µl) and soil-containing benzene (150 µl benzene in 0.5 g soil) in rats, the absorption fraction was 85% for pure benzene, 104% for sandy soil and 63% for clay soil (Turkall *et al.* 1988). These three forms of benzene appeared to be absorbed rapidly following ingestion, with a half-life time of 4 to 7 minutes. However, a small portion of benzene would be volatilized in the gastrointestinal tract before it was absorbed. Bioavailability could be enhanced by the administration of benzene on soil.

C. Dermal

Humans

Data from either *in vivo* or *in vitro* studies showed a slight absorption of benzene through the human skin. Most benzene tended to be rapidly volatilized.

Franz (1984) investigated the dermal absorption of benzene in four volunteers. Pure benzene liquid (0.4 ml) was applied to 80 cm^2 of forearm. The dermal bioavailability was determined by the measurement of urinary excretion. The bioavailability factor was 0.05% at 2 hour post-exposure. Maibach (1980a, 1980b) reported 0.13% and 0.07% of absorption in human palm and forearm skin, respectively. These exposures involved very high exposure levels because pure benzene was used.

With an *in vitro* human study, the degree of absorption through abdominal skin was proportional to the concentrations (5 to 520 $\mu l/cm^2$) of benzene applied on the skin (Franz 1984). The bioavailability factor remained constant (0.02%) at each concentration. Furthermore, the permeability of benzene varied with the carrier vehicles (Blank and McAuliffe 1985). The mean permeability constant was 111000 cm/h, 1400 cm/h, 900 cm/h, and 3700 cm/h for benzene in water, gasoline, hexadecane and isooctane, respectively.

Animals

Benzene is also slightly absorbed in animals. Results from an *in vivo* study revealed that monkey and mini-pig had bioavailability factors of 0.14%

and 0.09% (Franz 1984). Maibach and Anjo (1981) found that 0.17% of pure benzene and 0.08% of 0.36% petroleum distillate mixture could penetrate monkey skin. The absorption fraction of benzene in hairless mice was 0.89% (Susten *et al.* 1985), with a permeability of 0.619 cm/h (Tsuruta *et al.* 1989). Using sandy and clay soil-adsorbed benzene, Skowronski *et al.* (1988) and Abdel-Rahman and Turkall (1989) observed that the percutaneous penetration was lower when compared to pure benzene exposure. Pure benzene (300 µl) and soil-adsorbed benzene (300 µl benzene in 1 g soil) were administered to the abdominal skin of hairless mice covered by a glass cap. The extent of absorption was estimated from the degree of excretion in expired air, urine and feces, with a correction for volatilization during the soil treatment. The bioavailability factors were 20%, 16% and 19% for benzene in sandy soil, clay soil and pure benzene, respectively, at 12 hour post-exposure, while the bioavailability factors were 70%, 56%, and 99% for the above three forms at 48 hours post-exposure. This bioavailability was much higher than that from Susten *et al.* (1985) possibly because the test substance was applied under a glass cap to avoid volatilization, and bioavailability was determined with a correction for volatilization. Without the correction, the absorption fraction would have only been 6% and 12% for benzene in sandy and clay soil at 12 hours post-exposure (Brainard and Beck 1992). From an *in vitro* study (diffusion cell technique), the absorption fractions were 0.20% for monkey and 0.23% for mini-pig (Franz 1984).

D. Summary

Benzene is bioavailable in humans and animals by inhalation, ingestion and dermal contact (Table 6-9, 6-10, 6-11, and 6-12, Figure 6-4). The absorption of benzene through inhalation and ingestion is high, whereas absorption via dermal contact is extremely low unless methods to avoid the volatilization of benzene on the tested skin are used in the experiments.

6.4.4 Uncertainties

Benzene is a highly volatile compound. It is released to the air, water and soil with a short half-life. Atmospheric benzene is readily degraded by photochemical reaction. In water and soil, it is volatilized and biodegraded. Benzene is relatively stable in groundwater. Based on the characteristics of the environmental fate, benzene is unlikely to accumulate in air, surface water and soil. Benzene may be taken up by plants around waste sites, and may contaminate groundwater. Currently, data on the benzene contamination of plants and groundwater are limited at the former wood preserving sites.

The toxicity of benzene is associated with benzene metabolism. In general, the three hepatic metabolites, phenol, catechol and hydroquinone, are considered to play a key role in benzene carcinogenicity for humans. Using an external dose for cancer risk assessment involves a number of uncertainties. Thus, the identification and quantification of benzene metabolites are critical for human health risk assessment. Recently, internal dose which frequently refers to the total benzene metabolites formed has been used for reassessing benzene cancer risk (Cox and Ricci 1992). Physiologically based pharmacokinetic models (PBPK) have been developed for a quantitative analysis of the distribution and metabolism of benzene in various tissues in humans and small rodents (Beliles and Totman 1989, Paxman and Rappaport 1990, Travis *et al.* 1990, Bois *et al.* 1991a, 1991b, Bois and Paxman 1992, Woodruff *et al.* 1992). The models are used to predict human cancer risk arising from exposure to benzene. However, the models have some limitations, such as the need for a detailed physiological description of benzene kinetics and a lack of understanding of the exact role of each metabolite involved in benzene carcinogenicity.

6.4.5 Conclusions

Humans are extensively exposed to benzene by inhalation in both general and occupational environments. In particular, smoking is a primary source for indoor air contamination by benzene. Nevertheless, exposure to benzene by inhalation for residents living near the contaminated sites may be insignificant because of the short half-life for atmospheric benzene. The potential hazard posed by ingestion and dermal exposure is minor for the general population because of the generally low benzene levels in foods, drinking water and soil.

Benzene can be absorbed via the three exposure routes. The bioavailability factor for inhalation ranges from 47% to 60% at two hour post-exposure in humans. Usually, the extent of absorption of benzene inhaled appears to be high during the first few minutes of exposure. Quantitative data on the oral bioavailability in humans are not available. From animal experiments, the oral bioavailability factors are extremely high in small rodents (between 89% to 97%). When benzene is adsorbed to soils, the bioavailability is altered, depending on the characteristics of types of soils. Dermal absorption is very low (less than 1%) by direct application of benzene on human or animal skin. The bioavailability factors are elevated if means to control benzene volatilization from the skin are used.

6.4.6 Recommendations for Further Evaluation and Research

Benzene bioavailability via certain exposure routes has been well studied in humans or animals. Direct study on oral bioavailability in humans is limited. There is a need to establish a relationship for oral bioavailability between animals and humans.

Benzene has been accepted as a cancer agent in human leukemia by inhalation exposure as well as causing other toxic effects. These effects may be attributed to its metabolites on the target sites. Accordingly, there is a need to investigate the quantitative relationship between absorbed dose (parent benzene) and the quantity of its metabolites in the systemic circulation, and the relationship between active species and benzene carcinogenicity as well as toxicity. In such a case, physiologically based pharmacokinetic models will be useful.

Table 6-9 Respiratory Bioavailability of Benzene in Humans

Benzene form	Number of Subjects	Exposure method	Benzene concentration	Experimental method	Exposure duration	Post-exp. duration	[1] Total intake	Bioavailability factor	Ref.
Vapor	23	Oral-only	47-110 ppmv (150-350 μg/L)	Exhalation measurement	2-3 hr	5 min 1-2 hr	81% 50%	70-80% 50% (20-60%)	a
Vapor	4	Whole-body	100 ppmv (300 mg/m^3)	Exhalation measurement	1-4 hr	4 hr	-	50-60%	b
Vapor	6	Whole-body	52-62 ppmv	Exhalation measurement	4 hr	3 hr	30%	47%[2]	c

Adapted from a: Srbova *et al.*,1950; b: Hunter 1968; c: Nomiyama *et al.* 1974
(1): Total Intake = (Concentration in the inhaled air - concentration in the expired air)/ concentration in the inhaled air
(2): The result was calculated from total intake plus fraction of air excretion

Table 6-10 Oral Bioavailability of Benzene in Animals

Benzene form	Species	Administration methods	Benzene carrier	Benzene dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Liquid	Rabbit	Gavage	-	Single: 0.4 g/kg	Mass-balance	2-3 d	84-89%	a
Liquid	Rat Mouse	Gavage	Corn oil	0.5-150 mg/kg	Mass-balance	-	>97% >97%	b
Liquid	Rat	Gavage	-	150 µl	Mass-balance	2 hr	85%	c
Liquid	Rat	Gavage	Sandy soil Clay soil	150 µl in 0.5 g soil	Mass-balance	2 hr	104% 63%	c

Adapted from a: Parke and Williams 1953; b: Sabourin *et al*. 1987; c: Turkall *et al*. 1988

Table 6-11 Dermal Bioavailability of Benzene in Humans and Animals (*In vivo*)

Species	Benzene carrier	Exposure area	Exposure region	Benzene dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Human	-	80 cm^2	Forearm	0.4 ml	Urinary excretion	2-4 hr	0.05%	a
Human	-	-	Forearm	-	-	-	0.07%	b
Human	-	-	Palm	-	-	-	0.13%	c
Monkey Mini-pig	- -	55-75 cm^2	Back	0.5 ml	Urinary excretion	2-4 hr	0.14% 0.09%	a
Monkey	- distillate	12.9 cm^2	Forearm	50 µl	Urinary and fecal excretion		0.17% 0.08%	d
Hairless mouse		0.8 cm^2	Back	5 µl	Mass-balance	4 hr	0.89%	e
Hairless mouse			Whole-body	200-3,000 ppm	Mass-balance		0.619 cm/h	f
Rat	-	13 cm^2	Abdominal	300 µl	Mass-balance	12 hr 48 hr	19% 99%	g
	Sandy soil			300 µl benzene in 1 g soil		12 hr 48 hr	20% 70%	
	Clay soil					12 hr 48 hr	16% 56%	

Adapted from a: Franz 1984; b: Maibach 1980a (cited by Susten *et al.* 1985); c: Maibach 1980b (cited by Susten *et al.* 1985); d: Maibach and Anjo 1981; e: Susten *et al.* 1985; f: Tsuruta *et al.* 1989; g: Skowronski *et al.* 1988.

Table 6-12 Dermal Bioavailability of Benzene in Humans and Animals (*In vitro*)

Species	Benzene carrier	Exposure region	Type of skin	Dosage or concentration	Experimental method	Permeability x 10^3 cm/h	Bioavailability factor	Ref.
Human	Water	Abdominal	Full-thickness	2 µl	Diffusion cell	111		a
	Isooctane					3.7		
	Hexane					2.4		
	Gasoline					1.4		
	Hexadecane					0.9		
Human	-	Abdominal	Split-thickness	5-520 µl/cm^2	Diffusion cell		0.20%	b
Monkey	-	Back	Split-thickness	5-200 µl/cm^2	Diffusion cell		0.19% (5 µl/cm^2)	b
Mini-pig	-	Back	Split-thickness	5-200 µl/cm^2	Diffusion cell		0.23% (5 µl/cm^2)	b

Adapted from a: Blank and McAuliffe 1985; b: Franz 1984

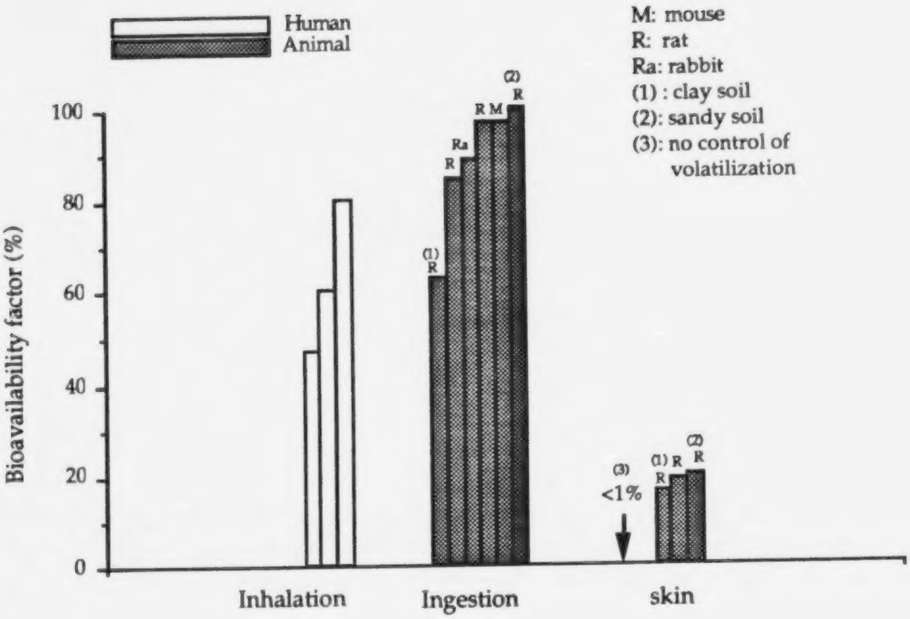

Figure 6-5 Bioavailability factors of benzene in humans and animals by three exposure routes [Adapted from sources in Table 6-9: a,b,c (inhalation); Table 6-10: a,b,c (ingestion); and Table 6-11: a-f (skin)].

6.5 Toluene

Toluene is a highly volatile, low water-solubility compound that is readily miscible with most organic solvents. The octanol/water partition coefficient (log k_{ow}) is 2.69 (Lide 1993).

Toluene readily partitions to air when emitted, with much smaller fractions partitioning to water and soil. Toluene rapidly degrades by reacting with hydroxyl radicals in air (WHO 1985, Howard 1990b). Most toluene in surface water and soil is readily volatilized to air (Howard 1990b). Also, toluene is rapidly biodegradable in surface water and soil, depending on microbial acclimation, temperature and mixing conditions. Toluene has been found in a few foods, such as baked potatoes, cheese, fried bacon, fried chicken, and fish (WHO 1985, Howard 1990b).

Toluene produces a range of toxic response in humans, including narcotic and neurotoxic effects (Ellenhorn and Barceloux 1988, ATSDR 1992).

Both general and occupational populations are primarily exposed to toluene through inhalation. The potential hazard via ingestion and dermal exposure is minor.

Bioavailability for toluene through all exposure routes is well studied in humans and animals. Toluene is completely absorbed in the gastrointestinal tract, while there is a relatively low dermal bioavailability.

6.5.1 Exposure Routes and Receptors

Humans may be exposed to toluene by inhalation. To a lesser extent, ingestion and dermal contact may be potential exposure routes.

In the occupational environment, workers in the printing industry, chemical manufacturing, and solvent operations are subject to toluene exposure by inhalation (Fishbein 1985, WHO 1985, ATSDR 1992). Dermal contact with toluene-based products (e.g. inks and dyes) is a significant exposure route for workers.

The general population may be exposed to toluene via inhalation indoors and outdoors. Toluene vapor may arise from emissions of automobile exhaust, gasoline and hazardous waste, cigarette smoke, toluene-containing products (paints, glue and cleaner) (Fishbein 1985, ATSDR 1992). Smoking is a major concern for exposure indoors of the general population to toluene (Hjelm *et al.* 1988, Brunnemann *et al.* 1989, Inoue *et al.* 1993). Because toluene generally occurs at low levels in drinking water, foods and soil, exposure by ingestion is unlikely to be significant (USEPA 1985b). People may be potentially exposed to toluene by dermal contact with toluene-based solvent, cosmetic products, and, to a limited degree, drinking water (Brown *et al.* 1984, WHO 1985).

6.5.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.5.3 on Bioavailability.

Distribution

In humans, toluene absorbed via inhalation is rapidly distributed in the blood, brain, liver, lung and fat tissue (Carlsson and Ljungquist 1982, Paterson and Sarvesvaran 1983, Takeichi *et al.* 1986, Ameno *et al.* 1989). The highest concentrations are found in the higher vascular tissue (e.g. brain and fat tissue) (Cohr and Stokholm 1979).

Metabolism and Excretion

About 70% to 80% of toluene absorbed is metabolized by the cytochrome P-450 mixed function oxidase system in the liver of humans (USEPA 1985b, WHO 1985, ATSDR 1992). The majority of toluene is converted to benzyl alcohol, which is subsequently catalyzed to benzoic acid by alcohol and aldehyde dehydrogenase. Benzoic acid is conjugated with glycine to produce hippuric acid. Also, the conjugation of benzoic acid with glucuronic acid forms benzoyl glucuronide. Small portions (less than 1%) of toluene are catalyzed to o-cresol and p-cresol by the cytochrome P-450 system, which finally react with sulfate glucuronide, glutathione or cysteine.

Approximately 20% of unchanged toluene is exhaled from the lung. Small amounts of parent compound and all the metabolites are primarily eliminated in urine. Hippuric acid is a major metabolite in urine, accounting for about 80% of the total excreted dose (Antti-Poika *et al.* 1987). Fecal excretion accounts for a very small fraction (less than 2%) of the total toluene excretion (Ellenhorn and Barceloux 1988).

6.5.3 Bioavailability

A. Inhalation

Respiratory bioavailability of toluene in humans is well-documented. As a vapor, toluene is readily absorbed following inhalation. In one study, the maximal blood toluene concentration was achieved after a two hour inhalation exposure (Sato and Nakajima 1978).

Because of the relatively low water solubility, the extent of the inhaled toluene depends on its deposition coefficient (percentage of total intake of the administered toluene) rather than the exposure concentration (Veulemans and Masschelein 1978). Once deposited in the mucous lining of the airway, it readily diffuses to blood because of the relatively high lipophilicity. In some cases, the bioavailability factors were estimated by using measurements of total intake of toluene in relation to the external dose. In fact, the concentration of toluene in alveolar air, reflecting the applied dose, is a more useful parameter for the evaluation of bioavailability. Bælum (1990) reported the averaged alveolar concentrations of toluene in humans only accounted for 18% and 19% of the total inhaled dose at rest and during exercise, respectively. However, there were large variations in alveolar concentrations of toluene among exposed individuals.

Respiratory bioavailability of toluene varies with the workload conditions in humans. Total intake was inversely proportional to workload during exposure (Carlsson 1982, Åstrand 1983). The bioavailability factors ranged from 39% to 51% at rest after two hour post-exposure, while the bioavailability factor decreased to 29% under extremely heavy work. In other studies, the bioavailability factor was between 37% and 51% (Nomiyama *et al.* 1974, Löf *et al.* 1990, 1993).

B. Ingestion

Humans

Human quantitative data on oral bioavailability of toluene are limited. In one human study, four volunteers were orally given a commercial toluene-containing food at a dose rate of 2 mg toluene/min for three hours (total 368 mg toluene) (Bælum *et al.* 1993). Hippuric acid in urine accounted for 87% to 100% of the total exposure dose. A hepatic extraction ratio (the ratio of the amount of toluene metabolized in the liver to the amount of toluene in the portal blood) was close to one (99% to 100%). This study indicated that almost all the toluene in the gastrointestinal tract was absorbed to the portal blood and metabolized in the liver. The fraction of parent toluene in the systemic circulation was close to zero.

Animals

A study with rats showed that the absorption rate of toluene via ingestion was slower than that via inhalation, with the maximal concentrations in various tissue occurring after 2 to 3 hour oral dosing (Pyykkö *et al.* 1977). When toluene was adsorbed to sandy and clay soil, the absorption rate in the gastrointestinal tract differed slightly from that via ingestion of pure toluene in rats (Turkall *et al.* 1990, 1991). All the three groups of toluene were readily absorbed to the portal blood. The fractions of urinary metabolites of toluene were 98%, 93% and 97% for pure, sandy and clay groups, respectively. These data reflected the high quantities of toluene in the portal blood.

C. Dermal

Humans

Following dermal exposure to toluene vapor, toluene appeared to be rapidly absorbed to the blood (Sato and Nakajima 1978, Riihimäki and Pfäffli 1978, Aitio *et al.* 1984). Dutkiewcz and Tyras (1968) investigated the quantitative absorption of toluene via dermal exposure in humans. The absorption rate was derived from indirect measurement, namely, the measurement of urinary metabolites, and the direct method, that is, the calculation of differences in the tested solution concentrations before and after dermal exposure by one hand immersion in the solution (Dutkiewcz and Tyras 1967). The absorption rate of toluene was relatively high (14 to 23 $mg/cm^2 h$). According to Fick's law, the permeability of liquid toluene was 0.9 cm/h in humans (Brown *et al.* 1984).

Animals

Dermal bioavailability has also been observed in animals. When hairless mice were exposed to toluene vapor at air concentrations of 200, 1,000 and 3,000 ppmv, the permeability was 1.24 cm/h (Tsuruta 1989). The bioavailability factor was 2.1% after toluene (5 μl) was directly applied to the back skin of hairless mice (Susten *et al.* 1990). Dermal bioavailability in rats tended to be higher by applying toluene on the skin with a glass cap to avoid the volatilization (Turkall *et al.* 1990). Absorption fractions estimated from blood, expired air, urine, and feces were 20%, 19% and 19% for toluene in pure, sandy and clay groups, respectively.

D. Summary

Toluene is bioavailable through all three exposure routes in humans and animals (Table 6-13, 6-14, 6-15, and Figure 6-6). Toluene in the gastrointestinal tract is almost completely absorbed to the portal blood. Respiratory bioavailability varies with rest and exercise conditions. Absorption via dermal contact is lower than that via other two routes, partially because of volatilization.

6.5.4 Uncertainties

Almost all the toluene released to the environment partitions to the air as a vapor phase. Toluene has a short half-life in the atmosphere. To determine adverse human health effects, specific information in relation to the atmospheric toluene levels is needed. However, available data at specific contaminated sites are limited. In general, residents living around the contaminated sites have a low level of exposure to toluene vapor by inhalation. With regard to the other environmental media, toluene has a short half-life in surface water or soil, but it can be relatively stable in groundwater or deep soil because of a slow biodegradation rate. There is no data on toluene levels in groundwater at the former wood preserving sites.

To date, the estimation of respiratory bioavailability in humans is based on the total intake of toluene. In effect, this parameter only represents the external dose. An attempt has been made to estimate the alveolar concentration of toluene recently. The internal dose of toluene in humans and animals is generally not available.

Active species and mechanisms of action for toluene are currently not fully understood. There are three postulated mechanisms, such as the alteration of the lipid structure of cell membrane by toluene, the interaction with cell protein by toluene, and the formation of the o-cresol and p-cresol intermediates (arene oxide intermediates) that may bind to cell protein or RNA (ATSDR 1992). Thus, toluene toxicity may be related to either the parent form or its metabolites. Although most absorption data via ingestion in humans and animals are derived from urinary metabolites, these values, as the upper bound of oral bioavailability factors, are useful for toluene risk assessment.

6.5.5 Conclusions

Exposure to toluene via inhalation is a significant route in comparison to the other routes for either the general or occupational population. Dermal exposure may occur in industries involving printing or using toluene as a solvent. The potential background risk via ingestion and dermal exposure is minor for the general population.

Toluene is readily absorbed by three exposure routes in humans. Respiratory bioavailability (based on the total intake) is variable with the workload rather than air concentration of toluene. The value is inversely proportional to the workload, ranging from 39% to 51% at rest and being 29% at heavy work following two hour post-exposure. The alveolar concentration (the applied dose) in humans is reported to be 18% to 19%. Toluene in the gastrointestinal tract is completely absorbed to the portal blood in humans and animals. For dermal exposure to toluene liquid or vapor, the permeability is 0.9 cm/h in humans. The bioavailability factor is 2.1% in hairless mice, and 20%, 19% and 19% in rats with dermal contact involving pure toluene, sandy and clay soil containing toluene, respectively, with control of volatilization.

6.5.6 Recommendations for Further Evaluation and Research

Because the evaluation of exposure to toluene vapor via inhalation depends on data relevant to the air concentrations, more specific information at the former wood preserving sites are required. Moreover, data on the toluene levels in groundwater are also needed if the drinking water is derived from groundwater near waste sites.

Currently, the estimation of respiratory bioavailability that is based on a total intake of toluene will overestimate the bioavailability. Only one study reported the alveolar concentration of toluene in humans. Thus, research to determine the applied and internal dose is needed.

Table 6-13 Respiratory Bioavailability of Toluene in Humans

Compound	Number of Subjects	Exposure method	Toluene concentration	Experimental method	Exposure duration	Post-exp duration	(1) Total intake	(2)Workload	Ref.
Vapor	10	Whole-body	98-130 ppmv (430 mg/m^3)	Exhalation measurement	4 hr	2 hr	37% (57%)(3)		a
Vapor	12	Whole-body	80 ppmv (300 mg/m^3)	Exhalation and blood measurement	30 min x 4	30 min 2 hr	55% 29% 32% 36% 39%	150 W 150 W 100 W 50 W Rest	b
Vapor	13	Whole-body	300 mg/m^3	Exhalation and blood	30 min x 4	30 min 1 hr 1.5 hr 2 hr	52% 52-55% 47-49% 50% 40% 50% 29% 40% 51%	50 W Rest 50 W Rest 100 W Rest 150 W 50 W Rest	c
Vapor	3 (patients)	Whole-body	3.25 mmol/m^3	Exhalation and blood measurement	4 hr	4 hr	51%		d
Vapor	9	Oral-nose	194 mg/m^3	Exhalation and blood measurement	2 hr	2 hr	51%	50 W	e

Adapted from a: Nomiyama *et al*. 1974; b: Carlsson 1982; c: Astrand 1983; d: Löf *et a*., 1990; e: Löf *et al*. 1993
(1):Total Intake = (Concentration in the inhaled air - concentration in the expired air)/ concentration in the inhaled air
(2): 50W = light work; 100W = moderate work; 150W = heavy work
(3): The result was derived from the sum of total intake and air excretion

Table 6-14 Oral Bioavailability of Toluene in Humans and Animals

Compound	Species	Administration methods	Toluene carrier	Toluene dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Liquid	Human	Feed-tube	tube feeding product	368 mg	Exhalation and urinary excretion	40 min x 4 (5 min break)	100%(1)	a
Liquid	Rat	Gavage	-	150μl	Mass-balance and urinary excretion	48 hr	98%(1)	b
Liquid	Rat	Gavage	Sandy Clay	150 μl toluene in 0.5 g soil	Mass-balance and urinary excretion	48 hr	93%(1) 97%(1)	b

Adapted from a: Bælum *et al*, 1993; b: Turkall *et al*. 1991
(1): the values are derived from the fraction of toluene metabolites in the urine

Table 6-15 Dermal Bioavailability of Toluene in Humans and Animals

Species	Compound and Carrier	Exposure region	Concentration and Dosage	Experimental method	Exposure duration	Bioavailability factor	Other	Ref.
Human	Liquid	One hand	100-600 mg/L	Loss in solution or urinary excretion	1 hr		Rate: 14-23 mg/cm^2 h Permeability: 0.9 cm/h	a b
Hairless mouse	Vapor	Whole-body	200 ppm 1,000 ppm 3,000 ppm	Mass-balance	6 hr		Permeability: 1.24 cm/h	c
Hairless mouse	Liquid	Back	5 µl	Mass-balance		2.1%		d
Rat	Pure	Abdominal	225 µl	Exhalation, blood, urinary, and fecal excretion	12 hr 48 hr	20% 79%		e
	Sandy		750 mg soil		12 hr 48 hr	19% 90%		
	Clay		750 mg soil		12 hr 48 hr	19% 81%		

Adapted from a: Dutkiewcz and Tyras 1967, 1968; b: Brown *et al*. 1984; c: Tsuruta 1989; d: Susten *et al*. 1990; e: Turkall *et al*, 1990

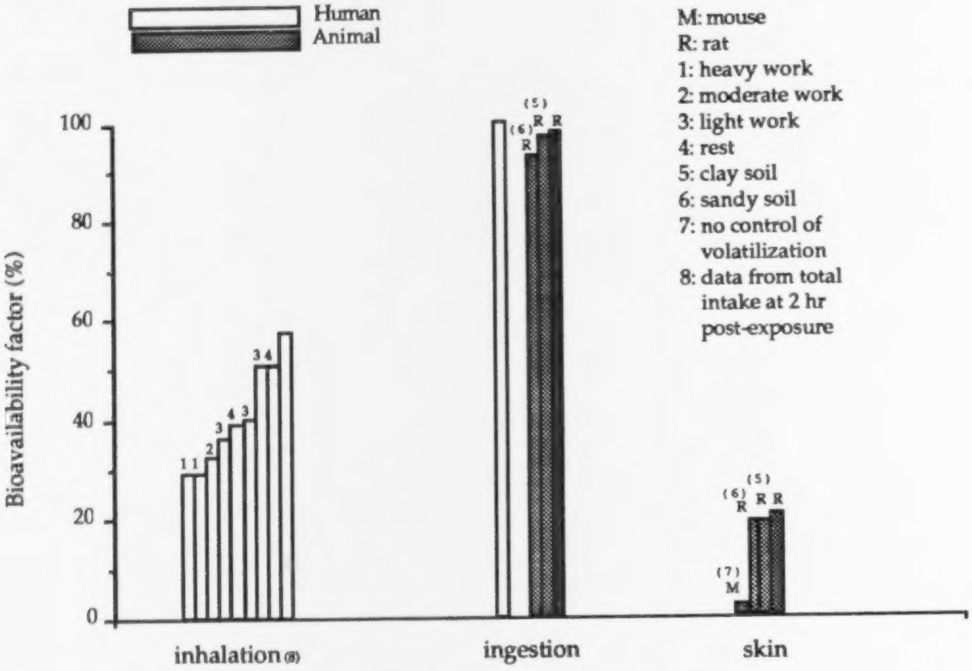

Figure 6-6 Bioavailability factors of toluene in humans and animals by three exposure routes Adapted from sources in Table 6-13: a-e (inhalation); Table 6-14: a,b (ingestion); and Table 6-15: d, e(skin)].

6.6 Polychlorinated Phenols: Pentachlorophenol, 2,4,5-Trichlorophenol, 2,4,6-Trichlorophenol, and 2,3,4,5-Tetrachlorophenol

Pentachlorophenol (PCP) is relatively volatile with very low water solubility. PCP is readily soluble in most organic solvents, such as benzene, acetone, ethanol and methanol and it has an octanol/water partition coefficient (log k_{ow}) of 3.32 at pH 7.2 (Lide 1993). Technical-grade PCP products include a variety of impurities, such as tetrachlorophenol, trichlorophenol, hexachlorobenzodioxins, hexachlorobenzofurans, and polychlorinated biphenyls (IARC 1991). 2,4,5-Trichlorophenol (2,4,5-T_3CP), 2,4,6-trichlorophenol (2,4,6-T_3CP), and 2,3,4,6-tetrachlorophenol (2,3,4,6-T_4CP) are also discussed in this section.

PCP is a widespread environmental pollutant that has been detected in the air, freshwater, fish and different foods. It mainly partitions into soil and the food chain when released. Commonly, pentachlorophenol occurs in the air as a vapor or aerosol that is directly emitted by vaporization from surface water, surface soil and treated-wood materials (USEPA 1979b, Geyer *et al*. 1986, WHO 1987). Information on ambient air levels for 2,4,5-T_3CP, 2,4,6-T_3CP and 2,3,4,6-T_4CP is limited. High levels of PCP in surface and groundwater have been extensively reported, particularly around wood treatment sites (WHO 1987, IARC 1991).

Almost all PCP (96.5%) released to the environment may partition into the soil, arising from direct soil application, volatilization, and wet deposition of atmospheric PCP (Hattemer-Frey and Travis 1989). PCP may be readily adsorbed to sediment (WHO 1987, IARC 1991). In principle, biodegradation occurs rapidly in soil under aerobic and anaerobic conditions for PCP and the other chlorophenol compounds (ATSDR 1989c, WHO 1989a, Lamar and Dietrich 1990, Seech *et al*. 1990). Roots of plants, rather than the upper part of plants, may absorb and accumulate PCP (Hattemer-Frey and Travis 1989). High PCP levels have been found in certain foodstuffs: grains, vegetables and fruits (Agriculture Canada 1989). Low levels of 2,4,5-T_3CP and 2,4,6-T_3CP are typically detected in fish, drinking water, and core or pea seedings (IARC 1979, WHO 1989a, ATSDR 1990b).

Toxic effects caused by PCP consist of skin injury, liver and renal function change, and central nervous disorders (IARC 1991). Adverse human health effects caused by 2,4,5-T_3CP, 2,4,6-T_3CP and 2,3,4,6-T_4CP are not well known. Because PCP is associated with the occurrence of hepatic cancer in mice by ingestion, it has been classified as a possible human carcinogen (McConnel 1989, IARC 1991). 2,4,5-T_3CP and 2,4,6-T_3CP are observed to produce cancer in mice but the data are currently inadequate to reach a conclusion on human carcinogenicity (IARC 1979, 1987).

PCP is bioavailable through inhalation, ingestion and dermal exposure in humans and animals. In particular, the absorption of the chemical in the gastrointestinal tract is almost complete. Bioavailability via inhalation is also high. From *in vitro* studies, PCP demonstrated a relatively high permeability through the human skin.

6.6.1 Exposure Routes and Receptors

Humans are exposed to PCP through inhalation, ingestion and dermal contact. Inhalation and dermal contact are primary exposure routes for workers, while ingestion is a major pathway for the general population.

PCP, 2,4,6-T_3CP and 2,3,4,6-T_4CP have been detected at high levels in the urine of workers (Gilbert *et al.* 1990, Pekari *et al.* 1991). The major sources come from wood treatment products and the other materials in the chemical industry (Exon 1984). Workers are primarily exposed to the chemical by inhalation (Casarett *et al.* 1969, Williams 1982, ATSDR 1990b). Alternatively, they have contact PCP on the skin during the handling of chemical products (WHO, 1987, 1989a, 1989b, ATSDR 1990b).

The general population may be exposed to PCP at low levels (Murphy *et al.* 1983, Cline *et al.* 1989, Hill *et al.* 1989). Ingestion of foods containing PCP is a primary source (Hattemer-Frey and Travis 1989). Exposure via ingestion of drinking water and soil appears to be less significant because of the low levels of PCP in water (WHO 1989b). Because there are increased levels of PCP in ambient air around waste sites or urban areas, the general population has a potential exposure to PCP via inhalation indoors and outdoors. However, a potential risk to the general population by dermal contact is judged to be insignificant.

The general population exposure to 2,4,5-T_3CP, 2,4,6-T_3CP and 2,3,4,6-T_4CP by ingestion of food and drinking water is likely to be significant (NRCC 1982, NHW 1988, WHO 1989a, ATSDR 1990b).

6.6.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.6.3 on Bioavailability.

Distribution

Once absorbed, PCP is distributed in various organs and tissues. It is highly protein-bound in plasma (Braun *et al.* 1977, Uhl *et al.* 1986). Free PCP in plasma reflects a recent exposure (ACGIH 1991). High levels of PCP have been found in the liver, kidney and brain in humans, and small amounts have been in fat, bone and muscle (Exon 1984, WHO 1987, IARC 1991). 2,3,4,6-T_4CP has been found in human liver tissue (Mussalo-Rauhamaa *et al.* 1989).

Metabolism

Studies from humans and animals have showed that PCP, 2,4,5-T_3CP, 2,4,6-T_3CP and 2,3,4,6-T_4CP were readily metabolized (WHO 1989a, Reigner *et al.* 1992a). They tended to conjugate with glucuronic acid. To a lesser extent, a small portion of PCP and 2,3,4,6-T_4CP undergoes oxidation to tetrachlorohydroquinone (TCHQ) (Ahlborg and Larsson 1978, Edgerton *et al.* 1979, Ahlborg and Thunberg 1980, Juhl *et al.* 1985).

The majority of PCP is protein bound in plasma. Both the unchanged forms and their metabolites are excreted in urine, and to a lesser extent in feces. From a review of human studies (Reigner *et al.* 1992a), the percentage of total pentachlorophenol-glucuronide (PCPG) recovered in urine ranged from 14% to 98.5%. This extreme variation has been attributed to the use of different analytical methods for PCP.

6.6.3 Bioavailability

A. Inhalation

From the occupational studies, PCP has been observed to be absorbed in humans (Casarett *et al.* 1969, Jones *et al.* 1986). Casarett *et al.* (1969) investigated the extent of absorption of PCP vapor by inhalation in two volunteers. Estimation of the bioavailability factor was based on the air concentration of PCP inhaled, the respiratory rate, and the tidal volume, with a correction of daily urinary excretion of the chemical. After dosing PCP at an air concentration of 230-432 ng/m^3, the bioavailability factor was 76% at 5 days post-exposure and 88% at 7 days post-exposure in two subjects, respectively.

Absorption of PCP via inhalation occurred in animals (Hoben *et al.* 1976). Rats inhaled sodium-pentachlorophenate (Na-PCP) aerosols at a single dose of 12.5 mg for 20 minutes. About 70-75% of the chemical was absorbed over a 24 hour period of exposure, by measuring the amount of PCP in the lung, liver, plasma and urine. Even though the rats received a continuous exposure for five days, the value of absorption was close to that from a single exposure.

No information was available with regard to the respiratory bioavailability for 2,4,5-T_3CP, 2,4,6-T_3CP and 2,3,4,6-T_4CP in humans and animals.

B. Ingestion

Humans

There was evidence indicating the absorption of PCP in humans via ingestion exposure (Braun *et al.* 1979, Uhl *et al.* 1986). The toxicokinetics of PCP were examined in four human volunteers (Braun *et al.* 1979). After the oral administration of sodium pentachlorophenate at a single dose of 0.1 mg/kg, absorption took place rapidly, with an absorption half-life of 1.3 hours and an absorption rate constant of 1.16 hr^{-1}. According to the extent of excretion of PCP in urine (74% of PCP and 12% of the conjugated PCP) and in feces (2% of both forms), the bioavailability factor was estimated to range from 74% to 98%. In another study, the half-life for absorption after the orally administered PCP was 16 days in human volunteers (Uhl *et al.* 1986).

Data on the oral bioavailability in humans for 2,4,5-T_3CP, 2,4,6-T_3CP and 2,3,4,6-T_4CP have not been reported.

Animals

A number of studies showed that PCP was well-absorbed in various animals. The results from studies revealed a high degree of absorption for PCP following oral administration, regardless of the species (monkeys, rats and mice), vehicles (e.g. corn oil, diet and water), and different doses (Braun and Sauerhoff 1976, Braun *et al.* 1977, Meerman *et al.* 1983, Reigner *et al.* 1991, 1992b). Rhesus monkeys were orally administered a corn oil containing PCP at a single dose of 10 mg/kg (Braun and Sauerhoff 1976). About 70% of unchanged PCP was present in urine, and 11% of the chemical was detected in tissues and carcass. Approximately 18% of PCP in feces was in the unchanged form. As a result, at least 81% of PCP was estimated to be absorbed. Similarly, the value in rats was 82% at a dose of 10 mg/kg and 64% at a dose of 100 mg/kg, by using the same experimental methods as in the monkey (Braun *et al.* 1977). In another three studies (Meerman *et al.* 1983, Reigner *et al.* 1991, 1992b), the bioavailability was determined by the comparison of the plasma concentration-time curve (AUC) via oral and intravenous administration. After receiving the PCP-containing water or diet, rats absorbed about 90% of the total administered dose over a 24 hour post-exposure period (Meerman *et al*. 1983). Reigner *et al*. (1991, 1992b) reported the oral bioavailability factor ranging from 91% to 97% in rats and 106% in mice.

Two studies revealed that over 80% of 2,4,6-T_3CP was absorbed in rats after the oral administration (Korte 1978, Bahig *et al*. 1981). The upper bound of values (from fecal excretion) ranged from 88% to 93%, while the lower bound values were between 82% to 94%.

C. Dermal

Few quantitative results of dermal bioavailability for PCP are available. Dermal absorption for PCP varies with the carrier vehicles. For *in vitro* human studies, the bioavailability factors were 16% with an aqueous vehicle, 62% with a diesel vehicle, 8% to 10% with an acetone vehicle and 15% to 19% with a soil (Horstman *et al*. 1989, Wester *et al*. 1993). The result from *in vivo* study in monkey revealed high bioavailability (Wester *et al*. 1993). With PCP-containing soil and acetone contact to the abdominal skin, 25% and 29% of the compound were absorbed into the systemic circulation over 14 days of exposure.

For *in vitro* studies, the permeability of 2,4,6-T_3CP through the human skin was 99,000 cm/min, and one through hairless mouse skin was 174,000 cm/hr at pH 5, 870 cm/hr at pH 6, and 41,000 cm/hr at pH 7.4 (Roberts *et al*. 1977, Huq *et al*. 1986).

D. Summary

PCP can be bioavailable through the three exposure routes (Table 6-16, 6-17, 6-18, and Figure 6-7). PCP is readily and rapidly absorbed via inhalation and ingestion in humans. Dermal bioavailability from *in vitro* human studies has been shown to be relatively high, depending on the types of vehicles.

2,4,6-T_3CP can be absorbed from the gastrointestinal tract and skin. Data on the bioavailability via all exposure routes for 2,4,5-T_3CP, and 2,3,4,6-T_4CP were not available.

6.6.4 Uncertainties

Humans may be exposed to PCP vapor or aerosol. Exposure to aerosols may be significant for residents around PCP contaminated sites because of the persistence of PCP in soil. Knowledge of particle size distribution and inhalable fraction for PCP are limited from either the literature or at specific sites. For respiratory bioavailability, the results were derived from two subjects who were exposed to PCP vapor. Nevertheless, data on deposition, retention and clearance of PCP aerosols are lacking in both humans and animals. The respiratory bioavailability for 2,4,5-T_3CP, 2,4,6-T_3CP and 2,3,4,6-T_4CP is also not clear, although these chemicals will generally become similar to PCP with respect to physicochemical properties.

The general population may be readily exposed to polychlorinated phenol compounds via ingestion of food, soil and drinking water. Quantitative results of PCP level in these media are still inadequate. The oral bioavailability in humans has been estimated from urinary and fecal excretion of PCP in four subjects. This sample number appears to be small. Because most values are derived from cumulative urinary and fecal excretion data in animals without quantification of their metabolites, these values are relatively unreliable.

A few quantitative studies were related to dermal exposure for these compounds. Dermal bioavailability in humans was determined by *in vitro* methods. There is not good agreement of these values between *in vitro* and *in vivo* experiments.

Humans may be generally exposed to mixtures of polychlorinated phenol compounds including PCP rather than pure PCP in the environment. These contaminants may alter the toxic profiles that are produced by pure PCP. For instance, dioxins and dibenzofurans are inducers of hepatic microsomal enzymes, some of which may enhance detoxification of PCP, so that PCP toxicity will be reduced (ATSDR 1989c). However, toxic effects arising from exposure to mixtures of polychlorinated phenol compounds including PCP may be attributed to other contaminants (e.g. dioxins and dibenzofurans) besides pure PCP. Bioavailability for technical-grade PCP has not been investigated.

6.6.5 Conclusions

Humans can be exposed to PCP through inhalation, ingestion and dermal contact. Inhalation and dermal contact are primary exposure routes for workers, while the exposure via ingestion is the primary route for the general population. Residents living around contaminated sites or in houses that used PCP-treated wood may be subjected to the higher levels of PCP by inhalation. Also, dermal contact with PCP-containing materials is a potential exposure route for the general population.

PCP is readily bioavailable via three exposure routes in humans and animals. The bioavailability factor for inhalation of PCP vapor is 88% over 7 days post-exposure in humans. For PCP aerosol, the bioavailability factor ranges from 70% to 75% in small rodents. The oral bioavailability in humans is estimated to be between 74% to 98% by the indirect method. However, PCP and 2,4,6-T_3CP in small rodents via ingestion are completely absorbed, being 64% to 100% for PCP and 82% to 94% for 2,4,6-T_3CP. The dermal bioavailability depends on the vehicles. By using *in vitro* methods, about 62% of PCP in diesel oil, 8% to 10% in acetone, 15% to 19% in soil and 16% in liquid can penetrate human cadaver skin. *In vivo* study indicates that the dermal bioavailability in monkey is 25% in soil and 29% in acetone. The permeability of 2,4,6-T_3CP through humans and hairless mouse skin is $9.9 \mathrm{x} 10^4$ cm/min and $2.9 \mathrm{x} 10^3$ cm/min, respectively.

Oral bioavailability for PCP appears to be only slightly affected by species differences, carrier vehicles, exposure levels and exposure duration. Absorption of PCP via ingestion is very high. Thus, exploration of methods to reduce absorption of PCP from the gastrointestinal tract will mitigete accidental exposures to PCP. For instance, an agent, cholestryramine, may enhance fecal excretion of PCP in monkeys, and may be recommended to use it for human overexposure to PCP (ATSDR 1989c).

Dermal bioavailability was found to be strongly influenced by different carrier vehicles and pH condition *in vitro*. Diesel oil and a low pH (acidic condition) will enhance the dermal absorption. Therefore, control of these factors may be reduce dermal absorption for PCP.

6.6.6 Recommendations for Further Evaluation and Research

For residents living around PCP contaminated sites, inhalation of PCP aerosols may be an important source of exposure. Data relevant to the bioavailability of PCP aerosols in humans and animals are critical for this assessment. These include inhalable fraction in air, particle size distribution, dissolution rate of soil-bound PCP, fractions of deposition, retention and mechanical clearance. For 2,4,5-T_3CP, 2,4,6-T_3CP, and 2,3,4,6-T_4CP, a study on the respiratory bioavailability is needed.

Information on the oral bioavailability for soil-bound PCP and dissolution rate in the gastrointestinal tract are required. An accurate assessment of oral bioavailability requires the use of blood measurements and the mass-balance technique.

A study on the correlation between *in vivo* and *in vitro* dermal bioavailability is needed.

Bioavailability by main exposure routes for technical-grade PCP and interaction among these contaminants need to be investigated.

Table 6-16 Respiratory Bioavailability of Pentachlorophenol in Humans and Animals

Compound	Species	Exposure method	Dose and concentration	Experimental method	Exposure duration	Post-exp duration	Bioavailability factor	Ref.
Vapor	Human	Whole-body	230-432 ng/m^3	Exhalation and urine measurement	45 min	5 d 7 d	76% 88%	a
Na-PCP Aerosol	Rat	Whole-body	Single: 12.5mg (0.1 mg/kg)	Mass-balance	20 min	24 hr	70-75%	b

Adapted from a: Casarett *et al.* 1969; b: Hoben *et al.* 1976

Table 6-17 Oral Bioavailability of PCP and 2,4,6-T$_3$CP in Humans and Animals

Compound & carrier	Species	Exposure method	Dose and Concentration	Experimental method	Exposure duration	Post-exp duration	Bioavailability factor	Ref.
Na-PCP	Human	Oral	Single: 0.1 mg/kg	Exhalation, blood, urinary and fecal excretion	-	8 d	98%$^{(1)}$ 88%$^{(2)}$ 74%$^{(3)}$	a
PCP in corn oil	Monkey	Oral	Single: 10 mg/kg	Mass-balance	-	15 d	81% 88%$^{(1)}$	b
PCP in corn oil	Rat	Oral	10 mg/kg 100 mg/kg	Mass-balance	-	7 d	82% 64%	c
PCP and Na-PCP in diet and water	Rat	Oral	350 ppm in diet and 288 mg/L in water	Blood measurement	7 d	24 hr	90%	d
PCP	Rat	Oral	Single: 2.5 mg/kg	Blood, urinary and fecal excretion	-	48 hr	91-97%	e
PCP	Mouse	Oral	Single: 15 mg/kg	Blood, urinary and fecal excretion	-	36 hr	106%	f

Adapted from a: Braun *et al*, 1979; b: Braun and Sauerhoff 1976; c: Braun *et al*, 1977; d: Meerman *et al*. 1983; e: Reigner *et al*, 1991; f: Reigner *et al*. 1992b

(Continued)

Compound & carrier	Species	Exposure method	Dose and Concentration	Experimental method	Exposure duration	Post-exp duration	Bioavailability factor	Ref.
2,4,6-T$_3$CP	Rat	Oral	1 ppm in diet	Urinary and fecal excretion	15 d	3 d	94%$^{(1)}$ 93%$^{(2)}$	g
2,4,6-T$_3$CP	Rat	Oral	1 mg/kg in diet	Mass-balance	3 d	5 d	88%$^{(1)}$ 82%$^{(2)}$	i

Adapted from g: Bahig *et al*. 1981; i: Korte 1978

$^{(1)}$: the values from fecal excretion data that are considered as the upper bound of bioavailability factor;

$^{(2)}$: the values from urinary excretion data , including the unaltered form and its metabolites;

$^{(3)}$: the values are the fraction of the unaltered form in urine.

Table 6-18 Dermal Bioavailability of PCP and 2,4,6-T_3CP in Humans and Animals

Species	Compound and Carrier	Exposure region	Skin area	Concentration and Dosage	Experimental method	Exposure duration	(1)BF and Permeability	Ref.
PCP								
Human	Liquid Diesel oil	Abdominal cadaver	1.0 cm^2	435 µg 1,540 µg	*In vitro* (diffusion cell)	24 hr	16% 62%	a
Human	Soil Acetone	Cadaver	1.0 cm^2	0.07 $\mu g/cm^2$ 0.8 $\mu g/cm^2$	*In vitro* (diffusion cell)	15 hr	15-19% 8-11%	b
Monkey	Soil Acetone	Abdominal skin	12 cm^2	0.07 $\mu g/cm^2$ 0.8 µg/cm2	*In vivo* (urinary excretion)	14 d	25% 29%	b
2,4,6-T_3CP								
Human	Solution	Abdominal cadaver	2.5 cm^2	-	*In vitro* (diffusion cell)	-	$9.9x10^4$ cm/min	c
Hairless mouse	Solution	Abdominal skin	-	0.05 g/ 100ml	*In vitro* (diffusion cell)	-	$174x10^3$ cm/h (pH 5) $87x10^3$ cm/h (pH 6) $41x10^3$ cm/h (pH 7.4)	d

Adapted from a: Horstman *et al.* 1989; b: Wester *et al*, 1993; c: Roberts *et al.* 1977; d: Huq *et al*, 1986
(1): BF =Bioavailability factor

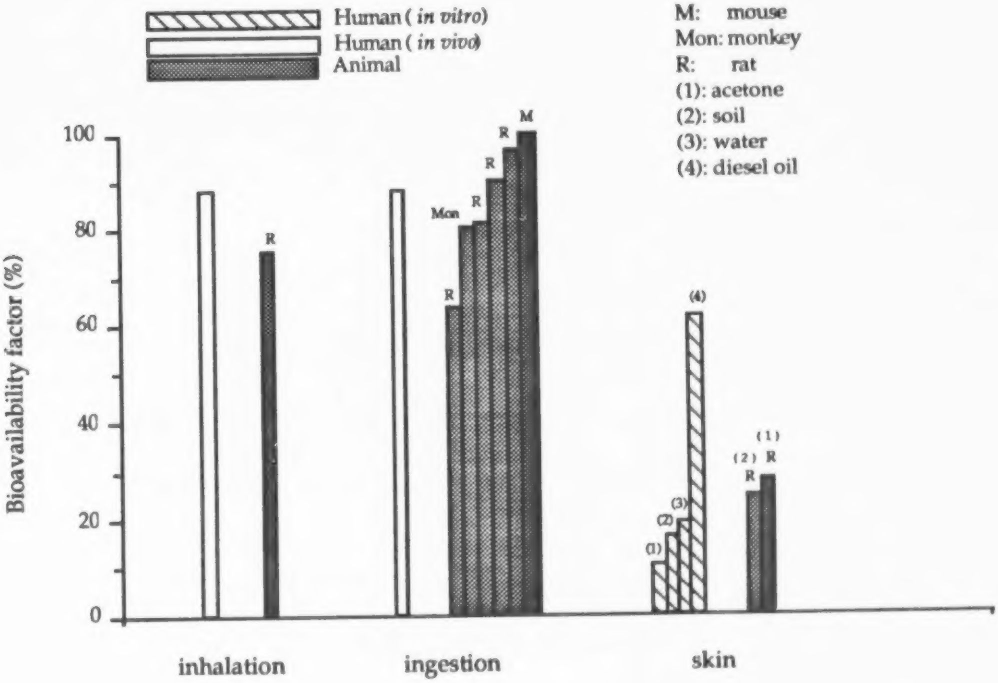

Figure 6-7 Bioavailability factors for pentachlorophenol in humans and animals by three exposure routes [Adapted from sources in Table 6-16: a,b (inhalation); Table 6-17: a-f (ingestion); and Table 6-18: a,b (skin)]

6.7 Polynuclear Aromatic Hydrocarbons (PAHs)

Polynuclear aromatic hydrocarbons (PAHs) are a class of organic chemicals consisting of multiple fused-benzene rings. The different physicochemical properties of PAHs, such as the number and orientation of rings, molecular weight, water solubility, volatility, result in the differences in their environmental characteristics, primary exposure routes and toxic effects. Some of PAHs that will be discussed in this section include:

Two-ring chemicals

- Naphthalene (volatile, slightly water-soluble)
- 2-methylnaphthalene (volatile)

Three-ring chemicals

- Acenaphthene (volatile)
- Anthracene (relatively water-insoluble)
- Carbazole (relatively water-insoluble)
- Fluorene (volatile)
- Phenanthrene (relatively water-insoluble)

Four-ring chemicals

- Chrysene (hydrophobic and low volatile)
- Fluoranthene (hydrophobic and low volatile)
- Pyrene (hydrophobic and low volatile)

Five-ring chemicals

- Benzo[a]anthracene (B[a]A) (highly hydrophobicv and non-volatile)
- Benzo[a]pyrene (B[a]P) (highly hydrophobic and non-volatile)
- Benzo[b]fluoranthene (B[b]F) (highly hydrophobic and non-volatile)
- Benzo[k]fluoranthene (B[k]F) (highly hydrophobic and non-volatile)

- Benzo[g,h,i]perylene (B[g,h,i]P) (highly hydrophobic and non-volatile)
- Dibenz[a,h]anthracene (DB[a,h]A) (highly hydrophobic and non-volatile)
- Indeno[1,2,3-c,d,]pyrene (highly hydrophobic and non-volatile)

PAHs have been detected in soil, sediment, water (ground water, surface water, drinking water and waste water) and air (IARC 1983, Menzin *et al.* 1992). Creosote vapors (naphthalene, 2-methylnaphthalene, acenaphthene, and fluorene) may be released to air by volatilization from the treated wood and creosote products (Heikkila *et al.* 1987, ATSDR 1990c, 1990d). Particulates containing PAHs have been observed in the atmosphere outdoors and indoors (IARC 1983, Menzie *et al.* 1992). Airborne PAHs can undergo photochemical degradation (ATSDR 1990e). Volatilized PAHs in surface water are subject to volatilization and photolysis. The water-insoluble PAHs and highly hydrophobic compounds tend to partition into organic matter in sediment. PAHs in soil may persist by adsorption to soil organic matter, giving rise to sorption, volatilization and biological or chemical degradation (Bulman *et al.* 1985). Some PAHs are detected in certain food groups, e.g. smoked meats, vegetables, grains, fats and oils (IARC 1983, ATSDR 1990e).

Toxic effects are incompletely studied for most PAHs despite the attention this group of compounds has received as environmental contaminants. Naphthalene and 2-methylnaphthalene can cause adverse health effects in the lung, blood and liver in humans (ATSDR 1990d). B[a]P has been reported to produce skin lesions in humans (ATSDR 1990f). Inhalation and dermal exposure to PAH-containing mixtures (e.g. coal tar, coke oven emission, soot and crude oil) in occupational settiings may be associated with an increased incidence of lung and skin cancer (ATSDR 1990e).

The following compounds are considered as possible human carcinogens or carcinogens in animals (IARC 1983): carbazole, chrysene, benzo[a]-anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, dibenz[a,h]anthracene, indeno[1,2,3-c,d,]pyrene. B[a]P can induce various tumors in animals through all exposure routes, so that IARC has classified B[a]P as a probable carcinogen to humans (IARC 1987). Other carcinogenic PAHs are primarily associated with skin cancer (IARC 1983).

Toxicity profiles may be altered with exposure to PAH-containing mixtures. Some PAHs components may serve as antagonistic or synergistic agents in animals. For instance, B[a]A was found to decrease the extent and rate of metabolism of B[a]P and B[a]P-DNA adduct formation in hamster embro cells, while anthracene may enhance the mutagenicity of B[a]P (ATSDR 1990e).

Limited information on the bioavailability is available for a few carcinogenic PAHs. Generally, B[a]P is well investigated with regard to its toxicokinetics. Hence, B[a]P is often used as a surrogate for carcinogenic PAHs.

6.7.1 Exposure Routes and Receptors

Inhalation and dermal exposure are primary pathways for workers using volatilized PAHs (ATSDR 1990e). The general population may be exposed to these compounds from smoking or when residents live near heavily PAH contaminated sites. High risk groups for exposure to PAHs include residents living in urban areas, or those who have nutritional deficiencies (e.g. iron, vitamins A and C), heavy smoking habits, genetic diseases (e.g. low DNA-repair capacities), and women in child-bearing age (maternal exposure) (ATSDR 1990e).

Because of the persistence of hydrophobic PAHs in soil, residents living around hazardous waste sites or urban areas have some potential exposure to airborne PAHs (e.g. engine exhaust and PAH-containing aerosols) via inhalation. Another important source is by active or passive inhalation of PAHs in tobacco smoke.

Exposure to PAHs by ingestion of drinking water and food is a potential exposure route of minimal significance for the general population because of the relatively low levels of PAH compounds in these media (IARC 1983). Carcinogenic PAHs have been commonly found in surface soils. Accordingly, exposure to soil-bound PAHs through ingestion may be a concern for the general population, particularly for young children with pica behavior (ATSDR 1990c, 1990e).

Dermal contact with PAH contaminated soil may be a significant pathway for residents living around PAH contaminated sites.

6.7.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.7.3 on Bioavailability.

Distribution:

B[a]P was found to be distributed in the lung, blood and intestines in rats after intratracheal administration (Weyand and Bevan 1986). Following oral exposure, B[a]P was distributed in the liver, lung and kidney in rats (ATSDR 1990e). A few studies have revealed that a very small fraction of PAHs was distributed to various organs and tissues.

Metabolism and Excretion:

Lung and skin have both been found to be capable of metabolizing B[a]P (Shinohara and Cerutti 1977, Boroujerdi *et al.* 1981, Wester and Maibach 1985, Noonan and Wester 1987, 1989). However, the liver was found to have a much greater capacity for B[a]P metabolism in comparison with the lung and skin.

Metabolism of B[a]P occurs in multiple steps in the liver (IARC 1983, Williams and Weisburger 1990). B[a]P is metabolized initially by cytochrome P-450 monooxygenase into arene oxides (e.g. 7,8-epoxide). Arene oxides may be catalyzed by epoxide hydrolase to form dihydrodiols before further conversion to diol epoxides (e.g. 7,8-dihydrodiol-9,10-epoxide). Arene oxides, dihydrodiols and diol epoxides may undergo conjugation with GSH, glucuronides, and sulfate esters. Alternatively, arene oxides and dihydrodiols can form phenols and phenol diols by rearrangement or hydrolysis. Of all the metabolites, diol epoxides are considered to be ultimate carcinogens which may be covalently bound to DNA and proteins.

B[a]P is primarily excreted via feces (biliary excretion) but it is usually metabolized prior to elimination through the bile duct (IARC 1983, ATSDR 1990f). Because of the hepatic presystemic metabolism, the biliary excretion rate could be enhanced following oral administration of B[a]P, and the distribution of B[a]P in tissues may be lower relative to other exposure routes (IARC 1983, ATSDR 1990f). Moreover, enterohepatic circulation has been observed for B[a]P and its metabolites (ATSDR 1990f).

6.7.3 Bioavailability

A. Inhalation

Humans

Some PAHs have been reported to be absorbed via inhalation exposure in humans. These compounds include naphthalene, chrysene, B[a]P, B[a]A, B[b]F, and DB[a,h]A (ATSDR 1990d, 1990f, 1990g, 1990h, 1990i, 1990k). Little is known of the quantitative bioavailability via inhalation.

Animals

Chrysene

Grimmer *et al.*, (1988) investigated urinary and fecal excretion of chrysene after intratracheal administration in rats. Based on cumulative urinary and fecal excretion, the respiratory bioavailability factor was estimated to be 53% and 41%, at single doses of 400 ng and 800 ng, respectively. About 16% of chrysene deposited within the respiratory tract was cleared to the gastrointestinal tract.

Benzo[a]pyrene

Deposition fractions in rats of pure B[a]P aerosols and B[a]P on ultrafine Ga_2O_3 particles (0.1 μm) were examined in rats by Sun *et al.* (1982). Following inhalation of both forms of B[a]P aerosols for 30 min, 20% of pure B[a]P aerosols and 10% of B[a]P on Ga_2O_3 particles were deposited within the respiratory tract. B[a]P on Ga_2O_3 particles was mainly cleared by mucociliary transport, while pure B[a]P aerosols appeared to be absorbed to the systemic circulation. The rate of mechanical clearance for B[a]P on Ga_2O_3 particles was slower (one day) than pure B[a]P aerosols (1.5 hours).

Pure B[a]P crystals and B[a]P-containing aerosols (0.1 to 1.0 μm and 15 to 30 μm) were intratracheally administered to mice (Cresia *et al.* 1976). The clearance rates of B[a]P from the lung varied depending on their aerosol size. About 95% of pure B[a]P crystals were cleared within 24 hours. Only 50% of B[a]P-containing aerosols between 0.1 to 1.0 μm were cleared from the lung within 36 hours.

The bioavailability factors, by intratracheal administration of B[a]P to rats, during 6 hour periods of exposure, ranged from 57% to 78% (Weyand and Bevan 1986, 1987). The values were 55% to 68% in guinea pig and 74% in hamsters (Weyand and Bevan 1987). When B[a]P on diesel carbonaceous particles was intratracheally administered to rats, the bioavailability factor declined to 20% after 6 hours dosing (Bevan and Ruggio 1991).

The bioavailability factors from isolated perfused rat lungs were highly variable (24% and 87%) (Foth *et al.* 1984, Törnquist *et al.* 1988). The highest value (87%) was obtained from experimental data with a correction of dissolution from air particulates and covalently bound DNA adducts (Törnquist *et al.* 1988).

B. Ingestion

Humans

Naphthalene and B[a]P were reported to be bioavailable via ingestion exposure in humans (ATSDR 1990d). Hecht *et al.* (1979) investigated oral absorption of B[a]P in humans. After ingestion of B[a]P-containing broiled meat (9μg B[a]P) by eight volunteers, only less than 0.1 μg of B[a]P was detected in feces. This result indicated that B[a]P was absorbed from the gastrointestinal tract. Quantitative data were not reported for other PAHs in humans.

Animals

Anthracene

Anthracene was intraduodenally administered to rats at a single dose of 1 mg in corn oil. During 24 hours post-exposure, the oral bioavailability factor was 71% (Rahman *et al.* 1986).

Chrysene

Grimmer *et al.* (1988) investigated the urinary and fecal excretion of chrysene after oral administration in rats. Based on cumulative urinary and fecal excretion, the oral bioavailability factor was estimated to be between 60% and 87%. The highest values from the fecal excretion represented the fraction of the compound in the portal circulation, as an upper bound value.

Phenanthrene

Phenanthrene was intraduodenally administered to rats at a single dose of 1 mg in corn oil. During 24 hours post-exposure, the oral bioavailability factor was 97% (Rahman *et al.* 1986).

Benzo[a]pyrene

B[a]P was intraduodenally administered to rats at a single dose of 1 mg in corn oil. During 24 hours post-exposure, the oral bioavailability factor was 23% (Rahman *et al.* 1986). When the rats were given a low dose of B[a]P in peanut oil (0.2 to 1 μg), the bioavailability factor was about 10% (Foth *et al.* 1988).

C. Dermal

Humans

Most PAHs have been observed to be absorbed through the skin in humans. These compounds included naphthalene, acenaphthene, anthracene, fluorene, fluoranthene, phenanthrene, pyrene, carbazole, chrysene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene, and dibenz[a,h]anthracene (Storer *et al.* 1984, ATSDR 1990d, VanRooij *et al.* 1993). The extent of absorption varied with anatomic sites and individuals (VanRooij *et al.* 1993). The dermal bioavailability factor in humans was estimated to range from 20% to 56% for PAHs following application of coal-tar ointment on various regions of the human skin at a low dose (e.g. about 1 ng pyrene/cm^2) (VanRooij *et al.* 1993). The results from *in vitro* studies indicated that the dermal bioavailability factor of B[a]P was about 24% to 31% in humans (Storm *et al.* 1990, Wester *et al.* 1990).

Animals

Anthracene

Anthracene dissolved in organic solvents was applied to the dorsal skin (2 cm^2) in rats. At 6 days, 52% of the chemical was recovered in the urine, feces and various tissues (Yang *et al.*, 1986a). Results from their *in vitro* experiment showed about 56% of the chemical penetrated the full-thickness skin (Yang *et al.* 1986a).

Phenanthrene

Dermal penetration of phenanthrene was studied by *in vivo* and *in vitro* methods in hairless guinea pig (Ng *et al.* 1991). About 80% of dermal absorption was observed from the *in vivo* method during 24-hour post-exposure. The *in vitro* penetration of the chemical was 71% to 78%.

Pyrene

Pyrene was studied in the hairless guinea pig by *in vivo* and *in vitro* methods (Ng *et al.* 1992). The bioavailability factor (*in vivo*) was 94% after 7 days dosing. The results from *in vitro* revealed about 40% to 69% of dermal penetration at the low and high doses during 24 hours exposure.

Dibenz[a,h]anthracene

DB[a,h]A dissolved in benzene was administered to the mouse skin (Heidelberger and Weiss 1955). At 2 day post-exposure, about 8% of the chemical was absorbed. Another study reported about 33% of the dermal absorption following dosing the chemical with acetone for 24 hours in mice (Sander *et al.* 1986).

Benzo[a]pyrene

The dermal bioavailability of B[a]P was well studied in animals by *in vivo* and *in vitro* methods (Kao *et al.* 1985, Sanders *et al.* 1984, 1986, Yang *et al.* 1986b, 1989a, 1989b, Storm *et al.* 1990, Wester *et al.* 1990, Ingram and Phillips 1993).

B[a]P was studied *in vivo* by application on the dorsal skin of animals with B[a]P dissolved in organic solvents (commonly acetone) at low and high doses. Bioavailability factors were determined after one day to one week post-exposure, by measurement of the cumulative biliary, urinary and fecal excretion data, or by using mass-balance technique.

In vitro studies were frequently conducted with a diffusion cell. A skin culture technique (i.e. viable skin) for determining the dermal absorption in various species was reported by Kao *et al.* (1985). The values from this technique were lower (3% in humans and 0.1% to 10% in small rodents) than those from the diffusion cell technique. The presystemic metabolism of B[a]P on the skin was thought to account for the apparent low dermal bioavailability.

There was relatively good agreement between *in vivo* and *in vitro* for the dermal bioavailability of B[a]P. In principle, the dermal bioavailability in animals was slightly higher than in humans. Values from a variety of studies varied according to the differences in species, applied dose and exposure duration. Results for B[a]P available in the literature are summarized as :

	In vivo	*In vitro*	Reference
Mouse	41%-83%	55%-60%	Sanders *et al.*1984, 1986; Ingram and Phillips 1993; Storm *et al.* 1990
Rat	35%-46%	38%-55%	Yang *et al.* 1986b, 1989a, 1989b; Storm *et al.* 1990
Hairless guinea pig	73%	40%-67%	Ng *et al.* 1992
Monkey	51%	-	Wester *et al.* 1990
Human	-	24%-31%	Storm *et al.* 1990; Wester *et al.* 1990

Mixtures

Because people are exposed to PAH-containing mixtures rather than isolated individual PAH chemicals in the environment, the study of bioavailability for the PAH-mixtures is crucial for quantitative risk assessment.

Cruzan *et al.* (1986) investigated the dermal bioavailability by *in vivo* and *in vitro* methods for some PAHs from clarified slurry oil in rats. The results indicated good agreements between *in vivo* and *in vitro* experiment for these hydrophobic compounds. The bioavailability factors were 48% (*in vivo*) and 44% (*in vitro*) for carbazole, 21% (*in vivo*) and 13% (*in vitro*) for four-ring PAHs (phenanthrene and fluorene), 1% (*in vitro*) for chrysene, 5% (*in vivo*) and 3% (*in vitro*) for five-ring PAHs (B[a]P), and 8% (*in vitro*) for 2-methylnaphthalene.

In another study, the pure B[a]P and a complex mixture of PAHs were applied to the mouse skin (Dankovic *et al.* 1989). B[a]P in the mixtures was more readily absorbed (16%) than the pure B[a]P (5%). The bioavailability factors were 16% to 19% for B[a]P, benzo[a]anthracene and benzo[b]fluorene, 13% for chrysene and 8% for pyrene.

D. Summary

B[a]P and other selected PAHs can be absorbed in humans and animals through all three exposure routes (Table 6-19, 6-20 and 6-21). The dermal bioavailability of B[a]P is well documented from *in vivo* and *in vitro* studies in either humans or animals (Figure 6-8, 6-9). The dermal bioavailability for each compound in the complex mixtures will be lower than pure chemical exposures. Pure B[a]P is more easily absorbed from the respiratory tract to the systemic circulation than B[a]P aerosols. The oral bioavailability of B[a]P appears to be lower in comparison to other exposure routes.

6.7.4 Uncertainties

The application of bioavailability for evaluating the PAH cancer risk is a complex issue, depending on the mechanism of carcinogenesis and exposure routes.

Lung tumors in animals are reported to be related to exposure to B[a]P via not only inhalation or intratracheal administration but also other exposure routes (IARC 1983, ATSDR, 1990f, Collins *et al.* 1991). Hence, the interaction between the B[a]P metabolites and DNA molecules may occur due to the metabolism of the deposited B[a]P within the respiratory tract or B[a]P and its metabolites transported from the systemic circulation via other exposure routes. B[a]P may also cause various tumors (including gastric tumors, pulmonary adenomas and leukemia) via ingestion (IARC 1983, ATSDR 1990f, Collins *et al.* 1991). The application of B[a]P on animal skin is observed to increase the incidence of skin cancer (IARC 1983, ATSDR 1990f, Collins *et al.* 1991). Consequently, both the quantitative analysis of DNA adduct formation and protein binding at the applied sites and internal dose (either the parent compound and its metabolites) are important for assessing cancer risk for PAHs.

Attempts have been made to determine quantitative data on the DNA and protein binding of B[a]P at the applied sites (Törnquist *et al.* 1988, Ingram and Phillips 1993). However, such information remains limited for most PAHs.

Because the occurrence of lung tumors was found to be correlated with B[a]P aerosols rather than pure B[a]P via inhalation, aerosols are considered to exert synergistic action in the development of lung tumors. An understanding of the characteristics of aerosols in air, deposition, retention and mechanical clearance within the respiratory tract will aid in assessing the cancer risk of PAHs. Parameters (inhalable fraction of B[a]P aerosols in the air and particle size distribution) that can be used to predict the deposition fraction are not available at specific contaminated sites. Data on the deposition, retention and clearance for B[a]P aerosols and other PAHs are limited.

Current quantitative values of respiratory bioavailability are obtained from the intratracheal administration of a few pure individual PAH compounds to animals. Such data will overestimate the human bioavailability for PAH-containing organic mixtures commonly found in the environment.

Oral bioavailability studies have been conducted for a few of the PAHs in animals. The values have been commonly determined by measurement of the extent of the biliary, urinary and fecal excretion. B[a]P is metabolized prior to elimination via the bile duct. As a result, the oral bioavailability factor usually consists of the fractions of the unaltered form and its metabolites that may come from the presystemic metabolism and systemic metabolism. To some extent, such results are likely to overestimate the oral bioavailability in human.

Quantitative studies on the dermal bioavailability in a great variety of species has been performed *in vivo* and *in vitro*. The skin absorption of B[a]P is dependent on the skin metabolism capacities as well as the physicochemical properties (Kao *et al.* 1985). Human data are only available *in vitro*. However, *in vitro* bioavailability does not account for the skin metabolism, so that such values tend to be an overestimation of bioavailability in humans.

Because most PAHs are hydrophobic, the chemicals released from the mixtures or solid carriers are most likely to easily penetrate lipophilic absorption barriers. Accordingly, determination of the physicochemical properties (octanol/water partition coefficients and dissolution rates under the physiological conditions) of PAHs is useful for predicting their bioavailability (Törnquist *et al.* 1988, Bevan and Ruggio 1991). Octanol/water partition coefficients are known for most PAHs.

Human exposure to PAH-containing mixtures may occur through all exposure routes. For mixtures, the absorption profiles can be affected by the interactions among individual chemicals. Results from the available experiments indicate lower dermal bioavailability after the application of the mixtures to the animal skins.

Dermal bioavailability, in some cases, has been found to be inversely related to the magnitude of the applied dose. Some studies showed high levels of PAHs applied on the skin were associated with high DNA adduct levels.

6.7.5 Conclusions

Humans can be exposed to PAHs from the environment through three exposure routes. Inhalation and dermal contact are the main exposure routes for the occupational population. Residents living near the PAHs contaminated sites may be exposed to airborne PAH particulates. Active or passive inhalation of PAHs from tobacco smoke is an important exposure source for the general population. Exposure to soil-bound PAHs via ingestion and dermal contact may be significant for high risk groups, particularly for young children with pica behavior. A potential risk for the general population may come from ingestion of contaminated food and drinking water. Water is considered to be minor exposure because of the low levels of PAHs usually detected.

PAHs can be absorbed in humans and animals via three exposure routes. Quantitative data on the respiratory and dermal bioavailability are available for a few of PAHs compounds. About 55% to 80% of B[a]P is bioavailable following intratracheal administration of the pure chemical in small rodents. The values decline to 20% to 40% for dosing of B[a]P-containing diesel particulates. Oral bioavailability tends to decrease with an increase of ring number of PAHs in animals: 97%, 70%, 60% and 10% to 30% for phenanthrene, anthracene, chrysene and B[a]P, respectively. *In vitro* dermal bioavailability for humans is between 24% and 30%. The *in vivo* and *in vitro* values from various animal species are relatively higher than ones from humans, ranging from 40% to 80%. The dermal bioavailability for individual chemicals through exposure to the PAHs-containing mixtures will markedly decrease.

Selection of bioavailability values for PAH risk assessment is generally dependent on the mechanisms of their carcinogenesis, specific exposure routes and specific environmental media. The lung cancer arising from exposure to some PAHs (especially, B[a]P) via inhalation and skin cancer via dermal contact with some PAHs appear to be local effects. In such cases, evaluation of deposition fraction for certain PAHs aerosols and determination of DNA or protein adduct formation at the applied sites (i.e. within the respiratory tract and skin surface) may be preferred for PAH risk assessment. For systemic carcinogenicity (e.g. via ingestion exposure), estimation of internal dose of PAHs is useful for risk assessment. Meanwhile, characteristics of the bioavailability values that are selected for calculation should be close to those characteristics that depict human exposure in the environment, such as specific exposure routes, carrier vehicles and ring number of PAHs.

6.7.6 Recommendations for Further Evaluation and Research

Further research on the bioavailability for PAHs needs to:

- determine the characteristics of the PAHs-containing aerosols from the contaminated sites, such as inhalable fraction of aerosols in the air, particle size distribution, and dissolution rates under similar physiological conditions.
- determine the profiles of deposition, retention and clearance for PAHs-containing aerosols or the mixtures.
- determine the respiratory bioavailability for each carcinogenic chemical or the PAHs mixtures by either *in vivo* and *in vitro* methods.
- determine the oral bioavailability for each carcinogenic chemical or the PAHs mixtures.
- identify and quantify the active species of carcinogenic PAHs in the systemic circulation that are administered via ingestion.
- determine the dissolution rates of soil-bound PAHs or the PAHs mixtures in the gastrointestinal tract.
- quantify the DNA adduct formation and protein binding at the applied sites.
- establish a correlation between *in vivo* and *in vitro* bioavailability for PAHs via inhalation and ingestion exposure.
- establish a relationship between the physicochemical properties (dissolution rates, molecular weight and octanol/water partition coefficients) and bioavailability for PAHs in order to predict the bioavailability by using *in vitro* methods.

Table 6-19 Respiratory Bioavailability of Some PAHs in Animals

Compound	Species	Exposure method	Dose or Concentration	Experimental method	Exposure duration	Post-exp duration	Bioavailability factor	Ref.
Chrysene	Rat	Intra-tracheal	Single: 800 ng Single: 400 ng	Urinary and fecal excretion	-	3 d 5d	41%(1) 53%(1)	a
B[a]P	Rat	Intra-tracheal	1 µg/kg	Mass balance	-	3 hr	57%	b
B[a]P	Rat	Intra-tracheal	0.16 µg 350 µg	Mass-balance	-	6 hr	78% 69%	c
B[a]P	Rat	Intra-tracheal	1 mg diesel particles	Mass-balance	-	6 hr 3 d	20% 40%	d
B[a]P	Hamster	Intra-tracheal	0.16 µg 350 µg	Mass-balance	-	6 hr	74% 74%	c
B[a]P	Guinea pig	Intra-tracheal	0.16 µg 350 µg	Mass-balance	-	6 hr	68% 55%	c
B[a]P	Rat	-	-	Isolated perfused lung	-	2 hr	24%	e
B[a]P	Rat	-	-	Isolated perfused lung	-	2.5 hr	87%(2)	f

Adapted from a: Grimmer *et al.* 1988; b: Weyand and Bevan 1986; c: Weyand and Bevan 1987; d: Bevan and Ruggio 1991; e: Foth *et al*, 1984; f: Törnquist *et al.* 1988

(1): Data from the sum of fraction of urinary excretion (both the unaltered form and metabolites) and fraction of fecal excretion (metabolites, excluding the unaltered form that may come from the respiratory mechanical clearance)

(2): Data with a correction of dissolution from urban air particulates and covalently bound DNA adducts.

Table 6-20 Oral Bioavailability of Some PAHs in Animals

Compound	Species	Exposure method	Dose and carrier	Experimental method	Exposure duration	Post-exp duration	Bioavailability factor	Ref.
Anthracene	Rat	Intra-duodenal	1 mg in corn oil	Biliary and urinary excretion	-	24 hr	71%	a
Chrysene	Rat	Gavage	50 μg in corn oil	Urinary and fecal excretion	-	4 d	87%(1) 60%(2)	b
Phenanthrene	Rat	Intra-duodenal	1 mg in corn oil	Biliary and urinary excretion	-	24 hr	97%	a
B[a]P	Rat	Intra-duodenal	1 mg in corn oil	Biliary and, urinary excretion	-	24 hr	23%	a
B[a]P	Rat	Gavage	0.2-1 μg in peanut oil	Blood measurement	-	24 hr	10%	c

Adapted from a: Rahman *et al*. 1986; b: Grimmer *et al*, 1988; c: Foth *et al*. 1988;
(1): Data from the cumulatively fecal excretion (the unaltered form) as the upper bound value.
(2): Data from the fecal excretion (metabolites) and the urinary excretion (both the altered form and metabolites).

Table 6-21 Dermal Bioavailability of Some PAHs in Humans and Animals (*in vivo* and *in vitro*)

Compound	Species	Carrier	Exposure region	Skin area	Concentration or Dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Anthracene	Rat	Acetone : hexane	Dorsal	2 cm^2	9.3 μg/cm^2	Mass-balance	6 d	52% (*in vivo*)	a
	Rat	Acetone : hexane	Dorsal	2.4 cm^2	9.3 μg/cm^2	Diffusion cell	6 d	56% (*in vitro*)	a
Phenanthrene	Hairless guinea pig	Acetone	Dorsal	4 cm^2	6.25 μg/cm^2	Urinary and fecal excretion	24 hr	80% (*in vivo*)	b
		Acetone	250 μm thick	1.7 cm^2	6.6 μg/cm^2 15.2 μg/cm^2	Diffusion cell	24 hr	78% (*in vitro*) 71% (*in vitro*)	
Pyrene	Hairless guinea pig	Acetone	Dorsal	4 cm^2	6.2 μg/cm^2	Urinary and fecal excretion	7 d	94% (*in vivo*)	c
			200 μm thick	2 cm^2	6.8 μg/cm^2 37 μg/cm^2	Diffusion cell	24 hr	69% (*in vitro*) 41% (*in vitro*)	
DB[a,h]A	Mouse	Benzene	Sacral	-	55 μg	Mass-balance	2 d	8% (*in vivo*)	d
DB[a,h]A	Mouse	Acetone	Dorsal	1.8 cm^2	5.4 μg/cm^2 56 μg/cm^2 515 μg/cm^2	Mass-balance	24 hr	82% (*in vivo*) 71% (*in vivo*) 33% (*in vivo*)	e
B[a]P	Mouse	Acetone	Dorsal	1.8 cm^2	1.25-12.5 μg/cm^2 125 μg/cm^2	Mass-balance	24 hr	83% (*in vivo*) 41% (*in vivo*)	e

Adapted from a: Yang *et al.* 1986a; b: Ng *et al*, 1991; c: Ng *et al.* 1992; d: Heidelberger and Weiss 1951; e: Sanders *et al*, 1984, 198;

(Continued)

Compound	Species	Carrier	Exposure region	Skin area	Concentration or Dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
B[a]P	Mouse	Acetone Mineral oil	Dorsal	5 cm^2	0.4 $\mu g/cm^2$	Urinary and fecal excretion	2 d	69% (*in vivo*) 82% (*in vivo*)	f
B[a]P	Mouse	Acetone	Dorsal	5 cm^2	2 $\mu g/cm^2$	Skin culture	24 hr	10% (*in vitro*)	g
B[a]P	Sencar mouse	Acetone	200 µm thick	0.64 cm^2	3 $\mu g/cm^2$	Diffusion cell	24 hr	55% (*in vitro*)	h
B[a]P	Balb/c mouse	Acetone	200 µm thick	0.64 cm^2	3 $\mu g/cm^2$	Diffusion cell	24 hr	60% (*in vitro*)	h
B[a]P	Rat	Acetone : hexane	Dorsal	2 cm^2	9.2 $\mu g/cm^2$	Mass-balance	5 d	46% (*in vivo*)	i
	Rat	Acetone : hexane	Dorsal	2.4 cm^2	9-10 $\mu g/cm^2$	Diffusion cell	5 d	50% (*in vitro*)	i
B[a]P	Rat	Acetone	Dorsal	5 cm^2	2 $\mu g/cm^2$	Skin culture	24 hr	2% (*in vitro*)	g
B[a]P	Osb-mendel rat	Acetone	200 µm thick	0.64 cm^2	3 $\mu g/cm^2$	Diffusion cell	24 hr	45% (*in vitro*)	h
B[a]P	Fuzzy rat	Acetone	200 µm thick	0.64 cm^2	3 $\mu g/cm^2$	Diffusion cell	24 hr	55% (*in vitro*)	h

Adapted from f: Ingram and Phillips 1993; g: Kao *et al*. 1985; h: Storm *et al*. 1990; i: Yang *et al*, 1986b

(Continued)

Compound	Species	Carrier	Exposure region	Skin area	Concentration or Dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
B[a]P	Rat	Crude Soil/crude	Dorsal	7 cm^2	0.09 mg/cm^2 9 mg/cm^2	Mass-balance	4 d	35% (*in vivo*) 9% (*in vivo*)	j
B[a]P	Rat	Crude Soil/crude Soil/crude Soil/B[a]P	350 μm thick		0.09 mg/cm^2 9 mg/cm^2 56 mg/cm^2 9 mg/cm^2	Diffusion cell	4 d	38% (*in vitro*) 8% (*in vitro*) 1% (*in vitro*) 1.7%-3.7%	j
B[a]P	Guinea pig	Acetone	Dorsal	5 cm^2	2 μg/cm^2	Skin culture	24 hr	0.1% (*in vitro*)	g
B[a]P	Hairless guinea pig	Acetone	Dorsal	4 cm^2	7.8 μg/cm^2	Urinary and fecal excretion	7 d	73% (*in vivo*)	c
			200 μm thick	2 cm^2	8.0 μg/cm^2 74μg/cm^2	Diffusion cell	24 hr	67% (*in vitro*) 40% (*in vitro*)	
B[a]P	Hairless guinea pig	Acetone	200 μm thick	0.64 cm^2	3 μg/cm^2	Diffusion cell	24 hr	58% (*in vitro*)	h
B[a]P	Rabbit	Acetone	Dorsal	5 cm^2	2 μg/cm^2	Skin culture	24 hr	1.5% (*in vitro*)	g
B[a]P	Marmoset	Acetone	Dorsal	5 cm^2	2 μg/cm^2	Skin culture	24 hr	3.5% (*in vitro*)	g
B[a]P	Monkey	Acetone Soil	Abdominal	12 cm^2	10 ppm 0.04 g soil /cm with 10 ppm B[a]P	Urinary excretion	24 hr	51% (*in vivo*) 13% (*in vivo*)	k

Adapted from j: Yang *et al*. 1989a and 1989b; k: Wester *et al*, 1990

(Continued)

Compound	Species	Carrier	Exposure region	Skin area	Concentration or Dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
B[a]P	Human	Acetone	Abdominal	0.64 cm^2	3 $\mu g/cm^2$	Diffusion cell	24 hr	31% (*in vitro*)	h
B[a]P	Human	Acetone	Leg	5 cm^2	2 $\mu g/cm^2$	Skin culture	24 hr	3% (*in vitro*)	g
B[a]P	Human	Acetone Soil	500 µm thick	1 cm^2	10 ppm/cm^2 0.04 g soil /cm with 10 ppm B[a]P	Diffusion cell	24 hr	24% (*in vitro*) 1.4% (*in vitro*)	k
Mixtures									
Carbazole	Rat	CSO(1)	Dorsal	1.8 cm^2	20 mg/cm^2	Mass-balance	4 d	48% (*in vivo*)	1
			350 µm thick	2.4 cm^2	20 mg/cm^2	Diffusion cell	4 d	44% (*in vitro*)	
2-methyl-naphthalene	Rat	CSO(1)	350 µm thick	2.4 cm^2	20 mg/cm^2	Diffusion cell	4 d	8% (*in vitro*)	1
Phenanthrene	Rat	CSO(1)	Dorsal	1.8 cm^2	20 mg/cm^2	Mass-balance	4 d	21% (*in vivo*)	1
			350 µm thick	2.4 cm^2	20 mg/cm^2	Diffusion cell	4 d	13% (*in vitro*)	
Fluorene	Rat	CSO(1)	350 µm thick	2.4 cm^2	20 mg/cm^2	Diffusion cell	4 d	13% (*in vitro*)	1

Adapted from 1: Cruzan *et al*. 1986
(1): CSO = Clarified Slurry Oil

(Continued)

Compound	Species	Carrier	Exposure region	Skin area	Concentration or Dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Chrysene	Rat	CSO(1)	350 µm thick	2.4 cm^2	20 mg/cm^2	Diffusion cell	4 d	1% (*in vitro*)	l
B[a]P	Rat	CSO(1)	Dorsal	1.8 cm^2	20 mg/cm^2	Mass-balance	4 d	5% (*in vivo*)	l
			350 µm thick	2.4 cm^2	20 mg/cm^2	Diffusion cell	4 d	3% (*in vitro*)	l
B[a]P	Mouse	Acetone	Dorsal	-	25 µg	Surface disappearance	24 hr	5% (*in vivo*)	m
B[a]P	Mouse	COM(2)	Dorsal	-	8 mg COM	Surface disappearance	24 hr	16% (*in vivo*)	m
B[a]A	Mouse	COM(2)	Dorsal	-	8 mg COM	Surface disappearance	24 hr	17% (*in vivo*)	m
B[b]F	Mouse	COM(2)	Dorsal	-	8 mg COM	Surface disappearance	24 hr	19% (*in vivo*)	m
Chrysene	Mouse	COM(2)	Dorsal	-	8 mg COM	Surface disappearance	24 hr	13% (*in vivo*)	m
Pyrene	Mouse	COM(2)	Dorsal	-	8 mg COM	Surface disappearance	24 hr	8% (*in vivo*)	m

Adapted from m: Dankovic *et al*, 1989

(2): COM = Coal-derived Complex Organic Mixture

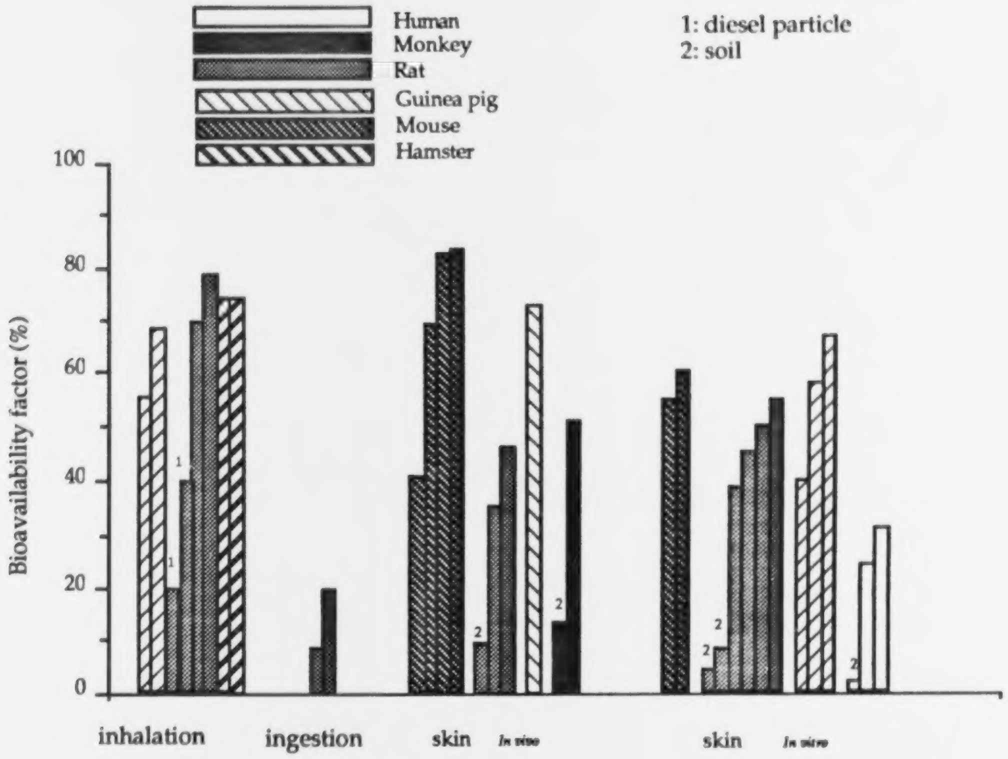

Figure 6-8 Bioavailability factors of benzo[a]pyrene in humans and animals by three exposure routes [Adapted from sources in Table 6-19: b,c,d(inhalation); Table 6-20: a,c (ingestion); and Table 6-21: c,e,f,g,i,j,k (skin)]

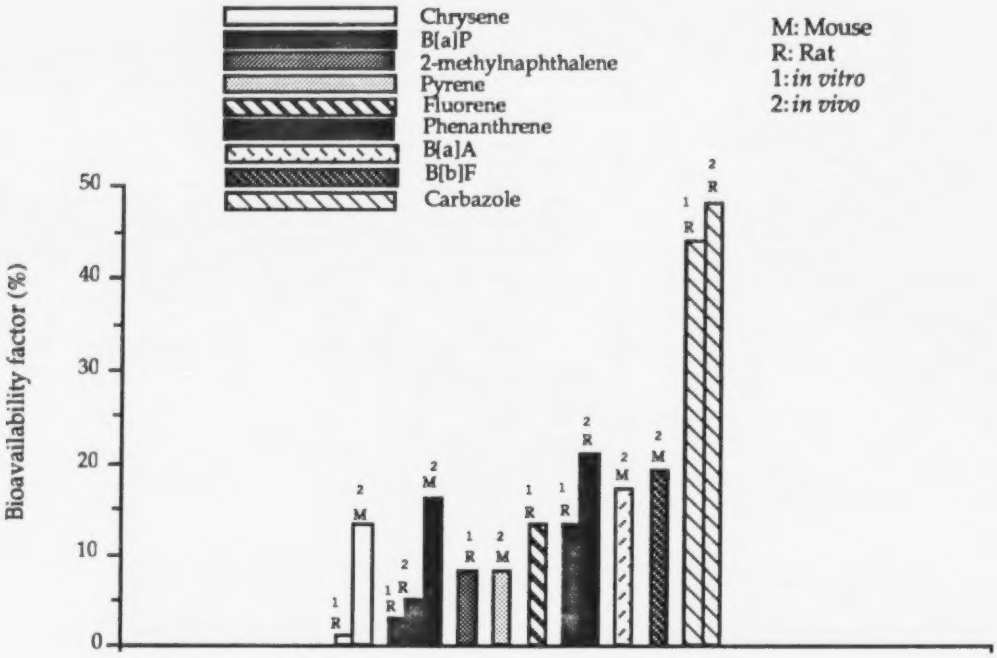

Figure 6-9 Dermal bioavailability factors for individual compounds after application of the PAHs-containing mixtures to animal skin [Adapted from sources in Table 6-21: l,m(skin)]

6.8 Phenol

Phenol is a colorless crystalline solid at room temperature, with an aromatic odour that is soluble in water and most organic solvents (Baich and Davist 1981, Ellenhorn and Barceloux 1988, Sittig 1991).

Phenol may be present in surface water, groundwater, soil and, somewhat, air from natural and anthropogenical (Bruce *et al.* 1987, Howard 1989a). Phenol exists in the air as a vapor phase and is rapidly removed by photochemical reaction (Howard 1989a). In surface water and soil, it is primarily biodegraded by microorganisms in a short time. Because phenol has a low adsorptivity to soil and a high mobility, it tends to leach to groundwater (ATSDR 1989d). Phenol has been detected in some foods, such as smoked summer sausage, fried bacon, fried chicken and mountain cheese (Howard 1989a).

Toxic effects caused by phenol include central nervous system depression with coma and respiratory arrest, diarrhea, sore throat, and chemical burn on skin (Ellenhorn and Barceloux 1988, ATSDR 1989d). No evidence has been found for carcinogenicity of phenol in humans and animals.

6.8.1 Exposure Routes and Receptors

Workers and residents living in vicinity of contaminated areas may be exposed to phenol vapor. However, information on air concentrations of phenol and the significance for this exposure route is limited.

For the general population, ingestion of foods and drinking water that are contaminated by phenol, or phenol products, may be a primary pathway (ATSDR 1989d). Also, dermal contact with waterborne phenol and phenol products can be significant. In terms of the characteristics of phenol in soil, inhalation of airborne phenol particulates and ingestion or dermal contact with the soil-bound phenol are unlikely to be significant routes for the general population.

6.8.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.8.3 on Bioavailability.

Distribution:

Phenol absorbed via ingestion was found to be rapidly distributed in the liver, lung, blood, brain, kidney, heart, spleen, adrenal and thyroid glands in animals (Deichmann 1944, Liao and Oehme 1982).

Metabolism

Most phenol can be conjugated with various acids to form phenylsulfate, phenylglucuronide, 1,4-dihydroxybenzene sulfate, and 1,4-dihydroxybenzene glucuronide in the liver or other organs (ATSDR 1989d). A small portion of phenol is oxidized to carbon dioxide, water, quinol (1,4-dihydroxybenzene) and catechol.

Phenol is mainly excreted in urine as sulfate or glucuronide conjugate forms and a small fraction in the unaltered form (Deichmann and Keplinger 1981). However, some phenol in urine may arise from the metabolism of aromatic amino acids (e.g. phenylalanine) or food components in the intestine.

6.8.3 Bioavailability

A. Inhalation

Phenol was observed to be absorbed via inhalation in humans (Piotrowski 1971, Ohtsuji and Ikeda 1972). Human volunteers inhaled a phenol vapor at the concentrations of 6-20 mg/m^3 for 8 hours (Piotrowski 1971). The mean retention fraction of phenol within the respiratory tract was 70%. About 84-99% of the retained phenol was eliminated in urine. Hence, the respiratory bioavailability factor was estimated to be 60-70% for humans.

The qualitative and quantitative information on the respiratory bioavailability for animals of phenol is lacking.

B. Ingestion

Phenol can be absorbed in the gastrointestinal tract in humans and various animal species (Capel *et al.* 1972, Kao and Bridges 1979, Edwards *et al.* 1986). Phenol liquid was orally administered to humans (0.01 mg/kg), rhesus monkey (50 mg/kg), and small rodents (25 mg/kg). By the use of the cumulative urinary excretion measurement, the oral bioavailability factor was 90% in humans, 44% in rhesus monkey, and 50% to 95% in small rodents after 24 hours exposure. Kao and Bridges (1979) reported only 0.5% of phenol that was orally administered to rats at a dose of 25 mg/kg was eliminated in feces, with 97% of urinary excretion during 24 hours post-exposure. In another study, 80% of the oral bioavailability factor was determined in rats following oral administration of a dose of 1.2 mg/kg phenol (Edwards *et al.* 1986).

The foregoing values consist of the fractions of the unaltered phenol and its metabolites. Commonly, the unaltered phenol only accounts for a very small part of the urinary excretion (Cassidy and Houston 1979).

C. Dermal

Piotrowski (1971) studied the dermal absorption in humans. Following whole-body exposure to phenol vapor, humans could absorb the vapor at a constant rate of 0.35 m^3/hr (this means that the quantity of phenol contained in 0.35 m^3 of air will penetrate the skin each hour). Thus, the degree of absorption of phenol vapor may be calculated by the product of the absorption rate constant and the phenol concentrations in air. Phenol aqueous solution was applied to the human forearm skin at various concentrations (Baranowska-Dutkiewicz 1981b). The dermal bioavailability factors ranged from 12% to 14% after 30 minutes exposure, and increased to 23% at one hour exposure. Feldmann and Maibach (1970) used phenol acetone solution to apply on the human forearm skin at a dose of 4 $\mu g/cm^2$. The dermal bioavailability factor was low (4.4%). Another study showed the dermal bioavailability in humans to be about 24% (Bucks *et al.* 1990).

Using *in vitro* studies, the dermal bioavailability factor for humans was reported to be 11% to 19% (Franz 1975, Hotchkiss *et al.* 1992). The *in vitro* values in rats were between 16% and 27% (Bronaugh and Stewart 1985a, 1985b, Bronaugh *et al.* 1982, Hotchkiss *et al.* 1992).

The dermal permeability through human skin was 0.8×10^3 cm/h (Roberts *et al.* 1977). The permeability in hairless and rats was higher than one from humans, being 20×10^3 cm/h and 11×10^3 cm/h, respectively (Roberts and Anderson 1975, Behl *et al.* 1983, Jetzer *et al.* 1986). Because phenol can cause skin damage, high concentrations of phenol applied on the skin resulted in the high dermal permeability in hairless mice (Behl *et al.* 1983).

D. Summary

Phenol can be readily and rapidly absorbed through inhalation, ingestion and dermal exposure routes in humans and animals (Table 6-22, 6-23, and

Figure 6-10). The dermal permeability is proportional to the applied phenol levels on skin.

6.8.4 Uncertainties

Bioavailability of phenol via three exposure routes has been extensively studied in humans and animals. Because phenol is primarily eliminated with the urine, most *in vivo* values are determined by the measurement of the cumulative urinary excretion. However, the majority of the excreted phenol occurs as metabolites in the urine. The significance of determining the parent compound or its metabolites relies on an adequate understanding of the mechanisms of phenol toxicity. The mechanism of phenol toxicity remains poorly understood. Recent *in vitro* studies indicate that the reactive metabolites (catechol, hydroxyquinol, p-benzoquinone and 4,4'-biphenol) of phenol may mediate phenol toxicity (ATSDR 1989d).

6.8.5 Conclusions

Humans may be exposed to phenol through three exposure routes. Ingestion of the phenol-contaminated foods and drinking water is a significant route for the general population. Exposure via inhalation of the phenol vapor may be a concern for workers and residents living near the contaminated areas containing phenol.

Phenol is bioavailable via three exposure routes. The respiratory bioavailability factor is 60% to 70% for humans. Absorption of phenol from the gastrointestinal tract into the portal blood is almost complete (90%). About 4.4% to 24% of the dermal bioavailability (*in vivo*) is reported in humans, while the dermal permeability through human skin is $0.8 \mathrm{x} 10^{3}$ cm/h.

6.8.6 Recommendations for Further Evaluation and Research

Bioavailability of phenol is well studied in humans. However, better understanding of the toxicity mechanism of phenol is needed for quantitative risk assessment.

Table 6-22 Bioavailability of Phenol in Humans and Animals (*in vivo*)

Compound	Species	Exposure method	Dose or Concentration	Experimental method	Exposure duration	Post-exp duration	Bioavailability factor	Ref.
Vapor	Human	Inhalation	6-20 mg/m^3	Urinary excretion	8 hr	8 hr	60%-70%	a
Liquid	Human	Oral	0.01 mg/kg	Urinary excretion	-	24 hr	90%(1)	b
Liquid	Rhesus monkey	Oral	50 mg/kg	Urinary excretion	-	24 hr	44%(1)	b
Liquid	Rabbit	Oral	25 mg/kg	Urinary excretion	-	24 hr	48%(1)	b
Liquid	Rat	Oral	25 mg/kg	Urinary excretion	-	24 hr	95%(1)	b
Liquid	Mouse	Oral	25 mg/kg	Urinary excretion	-	24 hr	66%(1)	b
Liquid	Hamster	Oral	25 mg/kg	Urinary excretion	-	24 hr	76%(1)	b
Liquid	Guinea pig	Oral	25 mg/kg	Urinary excretion	-	24 hr	64%(1)	b
Liquid	Rat	Oral	1.2 mg/kg	Urinary excretion	-	24 hr	80%	c
Liquid	Rat	Oral	25 mg/kg	Urinary excretion	-	24 hr	97%(1)	d

Adapted from a: Piotrowski 1971; b: Capel *et al*. 1972; c: Edwards *et al*. 1986; d: Kao and Bridges 1979

(Continued)

Compound	Species	Exposure method	Dose or Concentration	Experimental method	Exposure duration	Post-exp duration	Bioavailability factor	Ref.
Vapor	Human	Dermal (whole body)	5-25 mg/m^3	Urinary excretion	6 hr	6 hr	0.35 m^3/hr$^{(2)}$	a
Liquid (water)	Humans	Dermal (forearm)	0.3 mg/cm^2	Surface disappearance	-	1 hr 0.5 hr	23% 13%	e
			0.6 mg/cm^2			0.5 hr	14%	
			1.25 mg/cm^2			0.5 hr	12%	
Liquid (acetone)	Human	Dermal (forearm)	4 μg/cm^2	Urinary excretion	24 hr	5 d	4.4%	f
Liquid	Human	Dermal	-	-		-	24%	g

Adapted from e: Baranowska-Dutkiewicz 1981b; f: Feldmann and Maibach 1970; g: Bucks *et al* 1990

(1): Data from the cumulatively urinary excretion including the unaltered form and metabolites.

(2): Data from the absorption rate of 0.35 m^3/hr. The extent of absorption via dermal pre hour is calculated by the product of the air phenol levels and this rate constant.

Table 6-23 Dermal Bioavailability of Phenol in Humans and Animals (*in vitro*)

Species	Compound and Carrier	Exposure region	Concentration or Dosage	Experimental method	Exposure duration	BF and Permeability	Ref.
Human	Liquid	Abdominal cadaver	10 µl/cm^2	Diffusion cell	24 hr	11%	a
Human	Liquid	Breast	1.6-2.7 µg/cm^2	Diffusion cell	72 hr	19%	b
Rat	Liquid	Dorsal	1.6-2.7 µg/cm^2	Diffusion cell	72 hr	27%%	b
Rat	Liquid	-	-	Diffusion cell	-	16%	c
Human	Liquid	Abdominal cadaver	-	Diffusion cell	-	0.8x10^3 cm/h	d
Hairless mouse	Liquid	Dorsal and Abdominal	- 0.5 g/l 1.0 g/l 2.0 g/l 4.0 g/l 6.0 g/l	Diffusion cell	-	20.3x10^3 cm/h 22.7x10^3 cm/h 30.7x10^3 cm/h 46.6x10^3 cm/h 165.4x10^3 cm/h 202.7x10^3 cm/h	e
Hairless mouse	Liquid	Abdominal	-	Diffusion cell	-	18.8x10^3 cm/h	f
Rat	Liquid	Dorsal	-	Diffusion cell		11.4x10^3 cm/h	g

Adapted from a: Franz 1975; b: Hotchkiss *et al*. 1992; c: Bronaugh and Stewart 1985a,1985b; Bronaugh *et al*, 1982; d: Roberts *et al*. 1977; e: Behl *et al*. 1983; f: Jetzer *et al*. 1986; g: Roberts and Anderson 1975

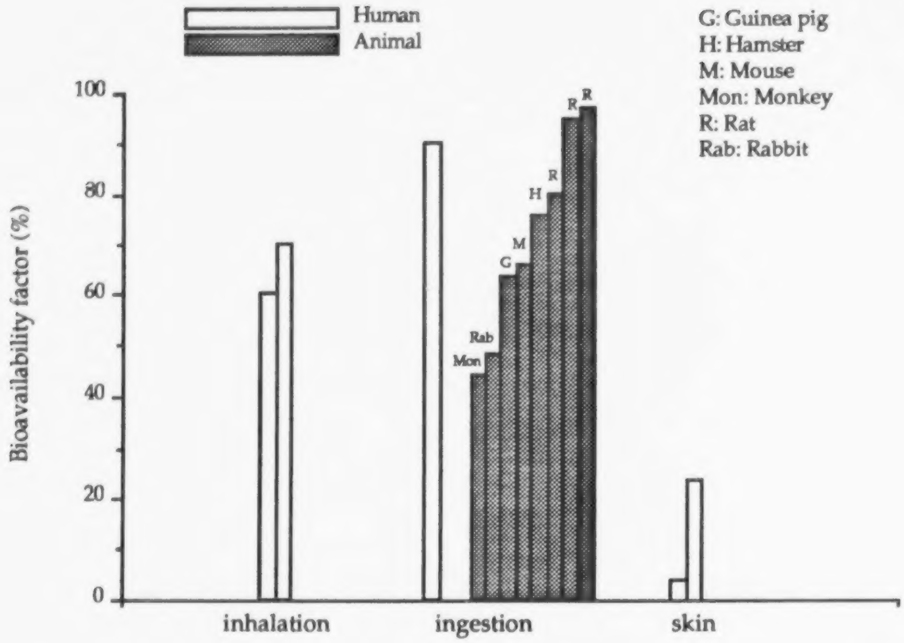

Figure 6-10 Bioavailability factors of phenol (*in vivo*) in humans and animals by three exposure routes (Adapted from sources in Table 6-22, a,b,c,d,f,g)

6.9 Aniline

Aniline is a volatile and oily liquid at room temperature that is very soluble in water and immiscible with most organic solvents (ACGIH 1991). Aniline has been detected in air, surface water, groundwater, soil, sediment, and some foods (IARC 1982b, Howard 1989b, ACGIH 1991). Aniline in air is degraded by photochemical reaction. In surface water, it is subject to biodegradation and photoxidation. A small part may be adsorbed to sediment. In addition to the biodegradation, aniline released to soil tends to be slightly or moderately adsorbed to organic matter. Aniline has been found in fresh fruits, vegetables, and salads.

Aniline is considered to be very toxic to humans with a lethal dose of 50 to 500 mg/kg (Sittig 1991). The characteristic of acute aniline toxicity is the blue skin arising from the formation of methemoglobin in the blood. Chronic toxic effects occur in the blood and on the skin. There is not sufficient evidence on the carcinogenicity of aniline in humans and animals (IARC 1982b).

6.9.1 Exposure Routes and Receptors

Occupational exposure to aniline via inhalation of vapor and dermal contact with vapor or liquid is significant (Howard, 1989b; and Sittig, 1991).

General populations may be exposed to aniline by ingestion of contaminated foods and drinking water. But a potential risk from this exposure route is minor because of the low levels of aniline present in these media. People may be exposed to aniline vapor from smoking or from the heavily contaminated air (IARC 1982b). A potential hazard from ingestion of soil-bound aniline or dermal contact with aniline-containing soil is unlikely to be significant for the general population.

6.9.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.9.3 on Bioavailability.

Distribution

Absorbed aniline is distributed throughout the body in animals, with the highest levels in the liver, blood, kidney and intestine (IARC 1982b). The distribution of aniline in humans has not been documented.

Metabolism and Excretion

In humans, aniline is primarily metabolized in the liver by hydroxylation in the para-position of the aromatic ring, and subsequently conjugated with sulfate and glucuronide to form the p-aminophenol conjugates (IARC 1982b, ACGIH 1991). N-hydroxylation of aniline also occurs to form phenylhydroxylamine, resulting in the formation of methemoglobin.

Aniline is mainly excreted by the urine. The extent of the urinary p-aminophenol has been found to be linearly proportional to the degree of absorption of aniline via inhalation and dermal exposure (Piotrowski 1957, Dutkiewicz and Piotrowski 1961). Thus, levels of the urinary p-aminophenol are used as an absorption index (35%) of aniline for humans (Piotrowski 1957). No phenyl-hydroxylamine has been observed in the urine in either humans or animals.

6.9.3 Bioavailability

A. Inhalation

The retention and absorption of aniline in humans have been investigated (Dutkiewcz and Piotrowski 1961, Dutkiewcz 1962a, 1962b). Following inhalation of the aniline vapor at the concentrations of 5-30 mg/m^3, about 90% of the inhaled aniline was retained within the respiratory tract. Because a very small portion of the inhaled aniline was mechanically cleared to the gastrointestinal tract, this value represented the respiratory bioavailability.

B. Ingestion

Aniline has been observed to be absorbed from the gastrointestinal tract in workers experiencing accidental ingestion (ACGIH 1991). Quantitative data are not available.

Freitage *et al.* (1982) orally administered aniline liquid to rats at a dose of 1 mg/kg for three days. The oral bioavailability factor was estimated to be 62% at 7 days post-exposure by using mass-balance technique.

C. Dermal

Aniline vapor or liquid can be absorbed via contact with the human skin (Piotrowski 1957, Dutkiewcz and Piotrowski 1961, Dutkiewicz 1961, 1962, Baranowska-Dutkiewicz 1982). Because of the existence of a linear relationship between aniline absorption and urinary p-aminophenol, the absorption profiles of aniline were obtained based on the degree of the urinary p-aminophenol (Piotrowski 1957). The absorption rate of aniline liquid through the human skin was reported to be 0.18 to 0.72 $mg/cm^2/h$ from the Piotrowski study (1957), and 2.0-4.6 $mg/cm^2/h$ from the Baranowska-Dutkiewicz study (1982).

Susten *et al.* (1990) investigated the dermal bioavailability of aniline through hairless mouse skin. About 4.7% of aniline was absorbed from the applied skin.

D. Summary

Aniline can be bioavailable via inhalation, ingestion and dermal exposure routes in humans (Table 6-24). The respiratory bioavailability is high in humans. The dermal absorption rate may be used to calculated the extent of dermal bioavailability for humans. Quantitative data on the oral bioavailability are limited for humans.

6.9.4 Uncertainties

Aniline is very toxic for humans. Generally, occupational populations may be exposed to high levels of aniline in air. Exposure to aniline vapor may be insignificant for residents living near specific contaminated sites. The adsorptivity to soil of aniline depends on the type of soil and soil pH condition. However, the data regarding the levels of soil-bound aniline are limited at the contaminated sites. Evaluation of exposure routes requires the specific information on the environmental profiles of aniline at the contaminated sites.

Bioavailability has been studied for human exposure to aniline vapor or liquid through inhalation and skin contact. The quantitative oral bioavailability, respiratory bioavailability of airborne aniline particulates and dermal absorption of soil-bound aniline is unknown.

6.9.5 Conclusions

Humans may be exposed to aniline via inhalation, ingestion and dermal contact. In general, occupational exposure to aniline vapor via inhalation and dermal contact is of concern. Potential exposure routes for general population may be related to the ingestion of the aniline-contaminated foods and drinking water, dermal contact with aniline liquid and inhalation of aniline vapor from smoking.

Aniline can be absorbed by inhalation, ingestion and dermal contact in humans and animals. The respiratory bioavailability is high (90%) in humans. The absorption rate of aniline through the human skin ranged from 0.18 to 4.6 mg/cm^2/h. About 62% and 4.7% of aniline are bioavailable via oral and dermal exposure, respectively, in small rodents.

6.9.6 Recommendations for Further Evaluation and Research

The following data is needed for quantitative risk assessment of aniline: the oral bioavailability in humans, the characteristic of aniline particulates near the contaminated sites, and the bioavailability for aniline aerosols via three exposure routes.

Table 6-24 Bioavailability of Aniline in Humans and Animals

Species	Compound and Carrier	Exposure method	Concentration or Dosage	Experimental method	Exposure duration	(1)BF and AR	Ref.
Human	Vapor	Inhalation	5-30 mg/m^3	Exhalation	-	90%	a
Rat	Liquid	Oral	1 mg/kg x 3 d	Mass-balance	7 d	62%	b
Hairless mouse	Liquid	Dermal	6.25 µl/cm^2	Mass-balance	4 hr	4.7%	c
Human	Liquid	Dermal (forearm)	0.25 ml in sponge patches	Surface disappearance	5 hr	0.18-0.72 mg/cm^2/h	d
Human	Liquid	Dermal (one hand)	-	(2)Urinary excretion	24 hr	2.0-4.6 mg/cm^2/h	e

Adapted from a: Dutkiewcz and Piotrowski,1961; and Dutkiewicz 1961,1962; b: Freitage *et al*. 1982; c: Susten *et al*. 1990; d: Piotrowski 1957; e: Baranowska-Dutkiewicz 1982

(1): BF = Bioavailability factor; AR = Absorption rate (dermal)

(2): The value is calculated based on the levels of the urinary p-aminophenol and absorption index of 35% that is derived from Piotrowski' study (1957)

6.10 Lead

Lead is a heavy bluish-gray, water-insoluble metal, having inorganic and organic forms (ATSDR 1991b, 1993c, Sittig 1991, Lide 1993). Some of its salts (nitrate, chlorate and acetate) are water soluble, and some lead salts (sulfate, chromate, phosphate and sulfide, and oxide) are insoluble in water. The nitrate, sulfide, and phosphate salts are soluble in acid. Toxicity of inorganic lead compounds is due to lead *per se* (Seiler and Sigel 1988). Lead can form stable organic compounds (tetra-alkyelead) that are soluble in most organic solvents but are insoluble water and decompose into free lead and organic radicals at high temperature.

Lead in the environment occurs naturally and anthropogenically. The lead levels in the environmental media are highly variable. Generally, lead tends to accumulate at discharge points (WHO 1977 1989c). Lead is found in air, soil, sediment, surface water, groundwater and biota. In surface water, lead is likely to form insoluble sulfates and carbonates (Carson *et al.* 1986). However, drinking water levels may be elevated by local dissolution in acidic and soft water (USEPA 1980c). Generally, lead is strongly adsorbed onto soil particles and sediment. Plants can be enriched in lead by atmospheric deposition and root absorption from soil (Seiler and Sigel 1988). The global average daily intake of lead estimated by United Nations Environment Program (UNEP) (1991) was 80 μg/d from food and 40 μg/d from drinking water.

Adverse human health effects produced by lead include anemia, kidney diseases, brain damage, impaired function in peripheral nerve system, high blood pressure in middle-aged men, reproductive abnormalities, developmental defects, abnormal vitamin D metabolism and even death under certain circumstances (ATSDR 1991b, 1993c). Low-level lead exposure may decrease intelligence quotient scores, impair the function of learning, attention and classroom performance, and reduce the growth rate of young children (Ellenhorn and Barceloux 1988, Demers 1991).

Lead can be absorbed via inhalation and ingestion. Organic lead compounds can easily penetrate the human skin.

6.10.1 Exposure Routes and Receptors

Humans may be exposed to lead via inhalation, ingestion and dermal contact in the occupational and general environments. Lead toxicity in adults mainly takes place in the occupational environment, while children can experience lead poisoning from nonoccupational exposure. High risk groups include young children (<6 years old), the elderly, pregnant women, smokers, alcoholics and people who have genetic diseases that are related to heme synthesis and other diseases (e.g. kidney dysfunction), and various nutritional deficiencies (e.g. zinc, iron, and calcium) (ATSDR 1993c).

Occupational populations may inhale airborne lead (dust and fume) during mining, smelting, refining and other operations at the workplace (Sittig 1991). Lead exposure also occurs via ingestion during eating or smoking in a lead-contaminated workplace (Baxter *et al.* 1985). Dermal exposure is much less significant than inhalation and ingestion. Only alkyl lead compounds and lead naphthenate in the occupational setting were reported to be slightly absorbed through the skin (Lauwerys 1983, ATSDR 1991b).

General populations may be exposed to airborne lead via inhalation of dusts or aerosols outdoors and indoors. However, direct inhalation of lead dusts generally accounts for only a small part of the total human exposure. Health and Welfare Canada estimated that about 23% of daily exposure for an urban adult and 1% to 2% for a child arising from daily inhalation (MOE 1987). Although inhalation is a minor exposure route for children, they may be more vulnerable than adults to exposure to airborne particles. Childern may exhibit higher deposition efficiencies for inhaled airborne particles than adults because of the physiological characteristics of their respiratory tract (Xu *et al.* 1986).

A potential hazard for the general population apparently arises from exposure to lead through ingestion of drinking water, food and various types of dusts or soils. Direct ingestion of soil/dust-bound lead, lead-containing paints and other lead-containing materials is a great concern for young children. Because lead has a long half-time in soils, lead soil or dust can become substantial contributors to blood lead for the general population. There is evidence indicating a positive correlation between soil lead from urban areas and blood lead (Steele *et al.* 1990). This type of relationship between soil lead and blood lead was not observed in mining sites.

General population exposure to inorganic lead by skin may be through the use of hair-darkening cosmetics (containing lead acetate). But this exposure route is generally considered to be insignificant because of the low dermal bioavailability of lead acetate in humans. However, Cohen and Rose

(1991) reported that human exposure to lead acetate from a hair coloring only accounted for about 0.5% of the total absorption of lead from the environment.

6.10.2 Toxicokinetics

Absorption: Discussion on the process of absorption is provided in Section 6.10.3 on Bioavailability.

Distribution:

The distribution of lead in the human body has been well characterized. In the simplified scheme, absorbed lead is distributed in blood, soft tissue and bone (Rabinowitz *et al.* 1976, Rabinowitz 1991). Once absorbed, 96% to 99% of blood lead is associated with the red cell (ATSDR 1991b, 1993c). About 80% of this erythrocyte pool is bound to hemoglobin, with a particular affinity for fetal hemoglobin, and the rest is bound to other protein, mainly albumin. A half-life for circulating lead is approximately one month (Rabinowitz *et al.* 1976).

Bone lead levels increase with age at rates dependent on the skeletal site and the degree of lead exposure. Approximately 95% of the total body burden is present in the bone of human adults and 73% of that is in the bone of children (Barry 1975). A half-life for bone lead is approximately 10 to 30 years.

Teeth can also accumulate lead. Tooth lead levels vary considerably with tooth types. Lead will accumulate in certain soft tissues, including brain, kidney, lungs, liver, heart and spleen (Barry 1975).

Metabolism and Excretion

Inorganic lead is not metabolized during phase I processes (such as oxidation, reduction and hydrolysis) but dose undergo phase II metabolic processes (biosynthetic reactions, such as conjugation with glucuronic acid and sulfate) (USEPA 1986). Alkyl lead compounds may be oxidized in the liver (Gerson 1990).

Lead is mainly eliminated via urine and feces. A "normal" value for lead in urine is ≤0.05 mg/L in the general population (ATSDR 1991b, 1993c). Exhalation of alkyl lead compounds following inhalation is a major route of elimination.

6.10.3 Bioavailability

A. Inhalation

Human exposure to lead often results from inhalation of lead aerosols. Prior to any evaluation of bioavailability, determination of the deposition of lead in the respiratory tract is crucial.

The deposition fraction of lead aerosols is considerably dependent on the particle size distribution. At a particle size less than 0.05 µm and inhalation rate of 15 breath/min, the deposition fractions of inhaled lead ranged from 35% to 70%, and decreased to 10% to 30% at a particle size between 0.05 to 0.5 µm (Booker *et al*. 1969, Hursh and Mercer 1970, Chamberlain *et al*. 1975, Wells *et al*. 1977, James 1978, Morrow *et al*. 1980, Gross, 1981, Chamberlain 1985).

Respiratory bioavailability studies have been conducted in human volunteers (Booker *et al*. 1969, Hursh and Mercer 1970, Chamberlain *et al*. 1975). In the studies from Booker *et al*. (1969) and Hursh and Mercer (1970), radio-labeled lead (^{212}Pb) obtained from thoron decay was administered to the volunteers by oral-only exposure. The absorption and retention of lead in the body was detected by means of the external whole body counting with a correction for lead levels in the blood and excreta. The absorption fractions of the deposited lead were 48% (24 hr) and 77% (72 hr). Wells *et al*. (1977) reported that about 50% of the deposited lead from exhaust aerosols was absorbed from the lung to the systemic circulation.

Tetraethyl lead from exhaust aerosols were inhaled by volunteers for 15 minutes. About 50% of the deposited lead and 5% of the ingested lead (swallowed to the gastrointestinal tract) were absorbed into the blood (Chamberlain *et al*. 1975). In another study, after exposure to tetraalkyl lead vapors, about 37% to 51% of the compounds were deposited within the human respiratory tract (ATSDR 1993c). About 68% to 80% of the deposited compounds were absorbed.

B. Ingestion

Isotope absorption studies in adults and lead balance studies in children have clarified the understanding of human lead bioavailability in the gastrointestinal tract. Absorption of lead occurs in the small intestine, and is affected by a variety of factors, such as age, solubility of lead salts and lead-containing soil or dust, nutritional status (feeding and fasting), dietary types (lipid content and milk), and dietary deficiency in the essential elements (iron, calcium, zinc, copper, selenium, chromium and manganese).

Generally, children tend to more easily absorb lead from the gastrointestinal tract than do adults. The metabolic balance technique was utilized to determine the oral bioavailability of lead for children (Alexander *et al.* 1974, Ziegler *et al.* 1978). Infant and young children were given lead with lead-containing food by feeding milk or formula and other foods. The daily intake, and urinary and fecal excretion data were recorded. The net absorption and retention of lead were calculated from these measurements. The oral bioavailability factors ranged from 41.5% to 53% for normal feeding status.

In adult studies, radio-isotope lead was orally administered to volunteers at various doses (single or multiple doses). The absorption and retention of lead in the blood were determined by whole-body counting, sometimes, with a correction of lead levels measured in the blood and excreta. In feeding studies, about 4% to 12.5% of lead ingested with meals was absorbed (Harrison *et al.* 1969, Rabinowitz *et al.* 1976, 1980, Blake *et al.* 1983, Chamberlain 1985). When the volunteers ingested the diet lead which was incorporated into foods, the bioavailability factor ranged between 3% to 10% (Rabinowitz *et al.* 1980, Heard and Chamberlain 1982, 1983). With slight fasting (light meal), about 16% to 21% of lead in water and 8.5% to 19% of lead in beverage (beer, tea and coffee) were bioavailable (Hursh and Suomel 1968; Blake 1976, Moore *et al.* 1979, Watson *et al.* 1980, Heard and Chamberlain 1983, Newton *et al.* 1992). In a fasting status, the oral bioavailability was markedly increased, ranging from 31% to 70% (Rabinowitz *et al.* 1980, Flanagan *et al.* 1982, Heard and Chamberlain 1982, 1983, Blake and Mann 1983, Chamberlain 1985).

Deficiency of calcium, vitamin D and iron, zinc, copper, phosphorus, dietary lipids, certain milk contents (particularly lactose) were reported to have very strong effects on lead absorption, resulting in an enhancement of lead absorption in humans (Heard and Chamberlain 1982, USEPA 1986, Chowdhury and Chandra 1987, ATSDR 1993c). A study showed that the bioavailability factor of lead in human volunteers declined from 69% without supplementation of any minerals to 10% with supplementation of calcium (200 mg) and phosphorus (140 mg) (Heard and Chamberlain 1982). Watson *et al.* (1980) found that iron deficiency resulted in elevated lead absorption in humans. But another study showed that there was no strong relationship between iron deficiency and increased lead absorption (Flanagan *et al.* 1982).

Numerous studies on oral bioavailability have been performed in animals (Forbes and Reina 1972, Garber and Wei 1974, Barltrop and Khoo 1975, Barltrop and Meek 1975, Barltrop *et al.* 1975, Quarterman and Morrison 1978, Quarterman *et al.* 1978, Pounds *et al.* 1978, Aungst *et al.* 1981, Baldini *et al.* 1989, Kapoor *et al.* 1989, Clapp *et al.* 1991, Dieter and Matthews 1993, Hayashi *et al.* 1993, Yannai and Sachs 1993). These studies, either qualitative or quantitative, indicated that lead absorption was also affected by age, nutritional status, dietary factors and carrier vehicles.

Lead in the environment often exists as a mixture of lead salts, soil/dust-bound lead or lead incorporated into a food matrix. The oral bioavailability depends on the solubility of these lead forms in the gastrointestinal tract. Some of lead compounds are easily dissolved in gastric acid. A study revealed that the solubility of lead salts increased as pH declined from pH 7 to pH 3; but as pH fell below pH 3, the solubility of lead decreased (Cheng *et al.* 1991).

In principle, the solubility in physiological condition of soil-bound lead from mining sites is lower than that from urban areas (Steele *et al.* 1990, Davis *et al.* 1992, Ruby *et al.* 1992). Davis *et al.* (1992) reported only 6% of mine-waste-impacted lead was dissolved in the rabbit small intestine. The low bioavailability of lead from mining sites is attributed to the low solubility of lead minerals.

C. Dermal

Absorption of inorganic lead compounds through the skin is much less significant than via other exposure routes because inorganic lead compounds have the ability to form complexes with proteins on skin to prevent the absorption (Hostynek *et al.* 1993). Organic lead compounds are lipid-soluble and easily bioavailable.

Lead naphthenate in lubricating oil was applied to human forearm skin for one hour at a dose of 32 µg/ml (Rasetti *et al.* 1961). The dermal permeability was 2×10^{-3} cm/h to 3×10^{-3} cm/h. In another study, the rate of absorption of lead acetate (containing in hair-darkening formulation) was reported to be 0.038 $\mu g/cm^2$ in 12 hours in the human volunteers (Moore *et al.* 1980).

With *in vitro* study, dermal bioavailability varied with the forms and speciation of lead (Bress 1991). The bioavailability factors were 6.3% for tetrabutyl lead, 1.3% for lead nuolate (oleate and linoleate), 0.3% for lead naphthenate and 0.05% for lead acetate.

In an animal study, tetraethyl lead was found to be rapidly absorbed through rat skin (Laug and Kunze 1948). The dermal bioavailability factor was approximately 6.5%, with the dermal permeability of 6×10^{-6} cm/h . With the application of lead acetate to rat skin, about 1.5% to 3% and 4.1% to 4.4% of the compound was absorbed in one week and eight weeks, respectively (Pounds 1979).

D. Summary

Lead can be rapidly bioavailable after inhalation and ingestion (Table 6-25, Table 6-26, and Figure 6-11). The deposition fraction of lead within the respiratory tract is inversely proportional to particle size distribution of lead aerosols. The oral bioavailability is influenced by nutritional status, age, lead species and forms, particle size and solubility of lead soil, and dietary types. Tetraalkyllead compounds more easily penetrate the human skin than inorganic lead compounds (Table 6-27).

6.10.4 Uncertainties

Generally, the respiratory and oral bioavailability are well characterized in humans. Recently, respiratory bioavailability factors have been derived from the deposited lead within the respiratory tract. Thus, the determination of deposition fraction of lead is critical for lead risk assessment. The estimation of deposition fraction requires information on the particle size distribution for lead in air at lead contaminated sites. However, such data are generally limited.

Most oral bioavailability studies are related to administration of isotope lead to humans. In reality, humans are exposed to lead with soil or dust, food and drinking water. The solubility of lead soil and dissolution rate of lead salts in the gastrointestinal tract determine the variability of oral bioavailability of lead. Such information at lead contaminated sites is not available.

The mechanisms of lead toxicity and interaction with other agents have not been clarified. Currently, a relationship between environmental lead levels and blood lead levels is commonly used for lead risk assessment. In some cases, environmental lead levels in certain media have not shown a linear relationship to blood lead levels (USEPA 1991b). One of the contributors for this nonlinear correlation may be due to different bioavailability of lead with different carrier vehicles of different lead forms.

6.10.5 Conclusions

The primary exposure routes for workers are through inhalation of lead dust and fume, ingestion of lead-contaminated food, and dermal contact with organic lead compounds. General populations are mainly exposed to lead via ingestion of lead contaminated food, drinking water and soil, and inhalation of lead soil or dust outdoors and indoors. Direct ingestion of lead soil and paint is a major potential risk for young children with pica behaviour. The potential hazard by dermal exposure to inorganic lead is negligible for the general population.

Inorganic lead compounds may be absorbed via inhalation and ingestion exposure. The respiratory bioavailability factors range from 48% to 77%. The deposition fractions are about 35% to 70% for lead aerosols less than 0.05 μm and 10% to 30% for ones between 0.05 to 0.5 μm. The oral bioavailability factors in a normal feeding status are 42% to 53% in children and 4% to 13% in adults, while the values increase to 30% to 70% in adults in a fasting status and 16% to 21% in a slight fasting with drinking water. Only 3% to 10% of dietary lead is bioavailable. Tetraalkyllead compounds can more readily penetrate through the skin (6.5%) than other lead compounds.

Lead absorption, especially in the gastrointestinal tract, is strongly affected by various factors. Thus, control of these factors will aid in reducing lead absorption and subsequently to reduce lead toxicity for humans. These control methods may include a supplementation of certain minerals (e.g. Ca, Fe, Cu and P) in daily diet and hygienic education for young children.

6.10.6 Recommendations for Further Evaluation and Research

In order to determine the deposition fraction of lead, the specific information on the particle size distribution and inhalable fraction of lead soil in air at the contaminated sites is needed. The solubility of lead-containing soil from the contaminated sites under the physiological condition are required to be studied. It is also important to determine the speciation of lead in water and dissolution of inorganic lead in the gastrointestinal fluid.

Table 6-25 Respiratory Bioavailability of Lead in Humans

Form or Compound	Carrier	Exposure method	Concentration or Dosage	Experimental method	Exposure duration	Bioavailability factor	Ref.
Inorganic							
^{212}Pb	Aerosol (0.05-0.5 μm)	Oral-only	1.6-1.6 μCi	Whole-body counting Blood and excreta measurement	72 hr	77% (1) (34%-60%)(2)	a
^{212}Pb	Aerosol (0.02-0.2 μm)	Oral-only	0.66-2.3 μCi	Whole-body counting Blood and excreta measurement	24 hr	48%(1) (44%)(2)	b
^{203}Pb	Exhaust aerosol (0.2-1 μm)	Oral-only	0.5-2.5 μCi	Whole-body counting Blood and excreta measurement	48 hr	50%(1) (35%)(2)	c
Organic							
Tetraethyl lead	Exhaust aerosol (0.2-1 μm)	Oral-only	0.5-5 mg/ m^3	Whole-body counting Blood and excreta measurement	72 hr	55%(1)	d
Tetraalkyl lead	Vapor	Oral-only	1 mg/ m^3	-	-	74% (1) (37%-51%)(2)	e

Adapted from a: Booker *et al.* 1969; b: Hursh and Mercer 1970; c: Wells *et al*, 197; d: Chamberlain *et al*, 1975 ;e: ATSDR 1993c

(1): data from the deposited dose of lead within the respiratory tract.

(2): deposition fraction

Table 6-26 Oral Bioavailability of Lead in Humans

Age (No. of subject)	Compound	Carrier	Nutritional status	Lead dose	Experimental method	Exposure duration	Bioavailability factor	Ref.
Adult(2)	$^{203}PbCl_2$	Meal	Fed	300 µg	Whole-body counting	4 d	4%	a
Adult(6)	$^{203}PbCl_2$	Meal	Fed	-	Whole-body counting Blood and excreta measurement	7 d	7.5%	b
Adult(4)	$^{204}Pb(NO_3)_2$	Meal	Fed	80 µg/d	Fecal excretion	20 d	8%	c
Adult(5)	$^{204}Pb(NO_3)_2$	Meal	Fed	79-204 µg/d (1-124 d)	Blood and excreta measurement	1-124 d	10%	d
Adult(3)	$^{203}PbCl_2$	Meal	Fed	5 µCi x 2 d	Whole-body counting Blood and excreta measurement	5 d	12.5%	e
Child((12)	Diet lead	Meal	Fed	30-130 µg/d (3 d)	Fecal and urinary excretion Lead intake	3 d	41.5%	f
Child(86)	Diet lead	Meal	Fed	1 µg/d x 3 d	Fecal and urinary excretion Lead intake	3 d	53%	g
Adult(6)	$^{203}PbCl_2$	Water	Light meal	10 µCi	Whole-body counting	12 d	16%	h
Adult(10)	$^{203}PbCl_2$	Water	Light meal	10 µCi	Whole-body counting	4 d	18%	i
Adult(11)	$^{203}PbCl_2$	Water	Light meal	-	Whole-body counting	-	21%	j
Adult(3)	$^{212}PbCl_2$	Beer	Light meal	5 µCi	Whole-body counting	2 d	8.5%	k

Adapted from a: Blake *et al*. 1983; b: Chamberlain *et al*, 1985; c: Rabinowitz *et al*, 1980; d: Rabinowitz *et al*, 1976; e: Harrison *et al*. 1969; f: Ziegler *et al*. 1978; g: Alexander *et al*. 1974; h: Watso 1980; i: Blake 1976; j: Moore *et al*, 1979; k: Hursh and Suomel, 1968

(Continued)

Age (No. of subject)	Compound	Carrier	Nutritional status	Lead dose	Experimental method	Exposure duration	Bioavailability factor	Ref.
Adult(9)	$^{203}PbCl_2$	Beer	Light meal	212 µg x 28 d	Whole-body counting Blood measurement	6 d	14%	l
Adult(4)	$^{203}PbCl_2$	Beer Tea/coffee	Light meal	1 µCi	Whole-body counting	2 d	19% 14%	m
Adult(6)	Diet lead	Meal	Fed	100 µg	Whole-body counting	7 d	3%	n
Adult(9)	Diet lead	Meal	Fed	1 µCi	Whole-body counting Blood measurement	2 d	5.5%	m
Adult(4)	Diet lead	Meal	Fed	80 µg/d	Fecal excretion	20 d	10%	c
Adult(4)	$^{204}PbSO_4$	Water	Fasted	79 µg/d	Fecal excretion	20 d	31%	c
	$^{204}Pb(NO_3)_2$	Water	Fasted	74 µg/d			37%	
Adult(6)	$^{203}PbCl_2$	Water	Fasted	1 µCi	Whole-body counting Blood measurement	2 d	44%	m
Adult(6)	$^{203}PbCl_2$	Water	Fasted	75 µg	Whole-body counting	-	45%	b
Adult(8)	$^{203}PbCl_2$	Water	Fasted	100 µg	Whole-body counting Blood measurement	7 d	60%	n
				plus Ca (200 mg) and P (140 mg)			10%	
Adult(85)	$^{203}PbCl_2$	Water	Fasted	-	Whole-body counting	15 d	60%	o
Adult(4)	$^{203}PbCl_2$	Water	Fasted	300 µg	Whole-body counting	4 d	70%	p

Adapted from l: Newton *et al*. 1992; m: Heard and Chamberlain 1983; n: Heard and Chamberlain 1982; o: Flanagan *et al*. 1982; p: Blake and Mann 1983

Table 6-27 Dermal Bioavailability of Lead in Humans and Animals

Species	Compound and Carrier	Exposure region	Skin area	Concentration and Dosage	Experimental method	Exposure duration	BF and Permeability	Ref.
Human	Lead naphthenate in lubricating oil	Forearm	-	32 ng/ml	-	1 hr	2-3 x 10^{-3} cm/h	a
Human	Tetrabutyl lead Lead nuolate Lead naphthenate Lead acetate	Abdominal	1.3 cm^2	10 mg	*In vitro* (diffusion cell)	24 hr	6.3% 1.3% 0.3% 0.05%	b
Rat	Tetraethyl lead	Dorsal	2.9 cm^2	106 mg	Mass-balance	24 hr	6.5%	c
Rat	Lead acetate	-	-	-	-	1 wk 4 wk 8 wk	1.6-3% 3.6-3.9 4.1-4.4%	d

Adapted from a: Rasetti *et al*. 1961 (cited by Hostynek *et al*. 1993); b: Bress 1991; c: Laug and Kunze 1948; d: Pounds 1979 (cited by Hostynek *et al*. 1993)

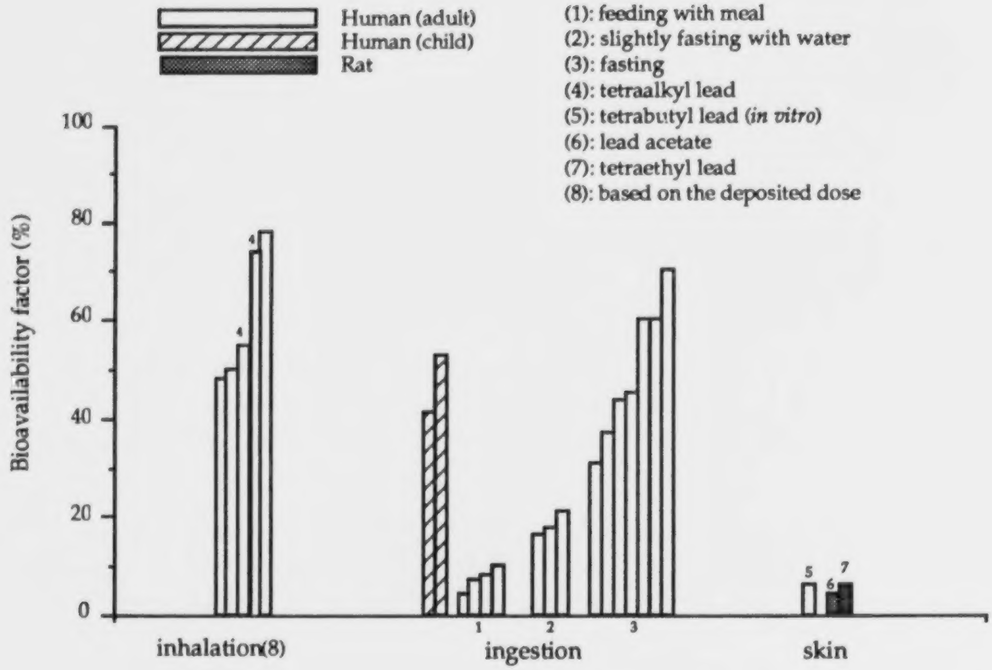

Figure 6-11 Bioavailability factors of lead in humans and animals by three exposure routes [Adapted from sources in Table 6-25, a-e (inhalation); Table 6-26, a-j, m-p (ingestion); and Table 6-27, b,c,d (skin)]

7. Discussion

The waste components from the former wood preserving sites in Alberta may contribute to a health risk by various exposure routes for populations living near the contaminated areas. Information on the bioavailability for the selected xenobiotics from the contaminated sites has been compiled and summarized from a through review of the scientific literature. The limited bioavailability data possess a relatively high level of uncertainty because of limitations inherent in the way they were derived. These limitations include: different experimental methods, the analytical capabilities, the species differences, knowledge of the specific mechanism of carcinogenicity, knowledge of the mode action of xenobiotics, the alteration of bioavailability caused by exposures to mixtures, variable chemical forms, and inadequate knowledge of exposure duration/frequency.

Identification of Exposure Routes

The wood preserving mixtures at the sites may be released into the surrounding environment. Whether and how these chemicals eventually enter the human body depends on their physicochemical properties and environmental fate, and their bioavailability.

Exposure to volatile compounds (such as benzene, toluene and creosote vapors) by inhalation is least likely to be significant for residents near the former contaminated sites because of the short half-life of these vapors in air. General populations would face greater inhalation risks from airborne particulates that adsorb high concentrations of xenobiotics such as chromium (VI), lead, copper, hydrophobic PAHs, hexachlorodibenzo[p]dioxin, and, possiblly, pentachloro-phenol.

Water-soluble compounds tend to easily partition into surface and ground waters. A hazard for residents may arise from exposure to these xenobiotics via ingestion of drinking water contaminated from the sites. Xenobiotics that are water soluble to some practical extent include chromium, arsenic, copper, lead, pentachlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, phenol, 2,4,6-trimethly-phenol, aniline, quinoline and two or three ring PAHs.

Local grown vegetables may also accumulate xenobiotics (e.g. pentachlorophenol, hexachlorodibenzo[p]dioxin, phenol, aniline and some PAHs) by deposition on leaves from vapors and aerosols or by absorption via roots from soil. Young children with pica behaviour are particularly susceptible to high levels of soil-bound xenobiotics, such as lead, pentachlorophenol, hexachlorodibenzo-[p]dioxin and some PAHs, becuase of their habit of ingesting soil.

Dermal exposure may be significant related to soil-bound hydrophobic PAHs, and to a lesser extent hexachlorodibenzo-[p]dioxin and pentachlorophenol. Occasionally, humans may be exposed to waterborne xenobiotics during swimming and bathing, but these exposures are judged to be rarely significant.

Overall, an evaluation of the significance of human exposure to xenobiotics through these exposure routes requires specific information on levels of xenobiotics and their stability in environmental media. Also, absorption profiles of xenobiotics are likely to affect the importance of different human exposure routes. For example, chromium species possesses a very low oral bioavailability in humans. Although humans could ingest a high level of chromium, the health risk may be relatively low. Likewise, human exposure to lead via dermal contact may be insignificant because of the low dermal bioavailability of lead. Potential routes for the general population exposure to xenobiotics at the former contaminated sites are summarized in Table 7-1.

Reliability and Validity of the Bioavailability Factors

The variability of bioavailability factor estimates for selected contaminants from former wood preserving sites are summarized in Table 7-2. A number of factors influence the reliability and validity of these estimates.

Choice of Experimental Methods. A great variety of experimental methods are involved in bioavailability studies. Each methodology offers both strengths and limitations, which inevitably result in overestimation or underestimation of true bioavailability factors.

Although the use of the blood measurement is reliable and accurate, it is not generaly practical for humans. Measurement of cumulative excreta data has been popular for human studies. In animal studies, a mass-balance technique has been common. Data derived from an accurate mass balance are more reliable than estimates from other experimented methods.

The most common method for determining the respiratory bioavailability in humans is the measurement of exhaled airborne compounds. Such values (e.g. bioavailability values for benzene, toluene and aniline) reflect the total mass of inhaled xenobiotics that are deposited within the respiratory tract. Using these values for estimating the respiratory bioavailability factors assumes complete absorption of the deposited compounds into the systemic circulation. Some bioavailability factors that are directly derived from the deposited dose (e.g. for arsenic and lead), if applicable, require a correction for the deposition fraction. Other bioavailability factors derived using the same methods, with a correction for urinary excretion or the deposition fraction (e.g. for pentachlorophenol and phenol) are more accurate and reliable.

For oral bioavailability studies, a wide range of the values has been observed. The upper bound values (by the measurement of cumulative fecal excretion data) often represent the total mass of the xenobiotics in the portal blood. The use of such values as the oral bioavailability factors makes an assumption that all the compounds in the portal blood enter the systemic circulation without undergoing hepatic presystemic metabolism. Lower bound values (by the measurement of cumulative urinary excretion data) account for a portion of the absorbed mass of the xenobiotics.

Some estimates are based on data including parent xenobiotic and its metabolites. Moreover, some studies are incomplete, without using a reference dose to eliminate the effects of metabolism and distribution for xenobiotics. In general, the oral bioavailability factors based strictly on the measurement of excreta data are unlikely to be reliable and accurate. However, true values likely fall between the lower and upper bound of estimates. This range of values still gives us insight into the absorption profiles for most xenobiotics.

In dermal studies, in addition to the bioavailability factor, the dermal permeability (occasionally, the absorption rate) is often determined. This parameter is suitable for assessing the internal dose by dermal exposure to vapors and waterborne xenobiotics. Estimates from surface disappearance techniques appear to be higher than ones from cumulative urinary data.

Both *in vivo* and *in vitro* techniques are commonly employed for dermal bioavailability studies, and, to lesser extent, for the respiratory bioavailability studies. *In vitro* data may differ with *in vivo* data for most xenobiotics. In some cases, *in vitro* data may be used to predict *in vivo* data if there is a good agreement between *in vivo* and *in vitro* for the similar xenobiotics. For some hydrophobic PAHs (carbazole, phenanthrene, anthracene and benzo[a]pyrene), a good correlation of the dermal bioavailability factors between *in vivo* and *in vitro* estimates has been found.

Alternatively, human *in vivo* values may be estimated from *in vitro* human data and from animal data on *in vivo* and *in vitro* . A relationship for dermal bioavailability has been proposed by USEPA (1992b):

$$\text{BF (Human } \textit{in vivo} \text{ estimate)} = \frac{\text{Human } \textit{in vitro} \text{ BF} \times \text{Species A } \textit{in vivo} \text{ BF}}{\text{Species A } \textit{in vitro} \text{ BF}}$$

Application of this equation assums that the ratio between *in vivo* and *in vitro* rates is the same for human and animal skin. For example, *in vitro* values of B[a]P in humans ranged from 24% to 31% (Storm *et al.* 1990, Wester *et al.* 1990). The data in rats are 46% *in vivo* and 50% *in vitro* (Yang *et al.* 1986b) Thus, the *in vivo* dermal bioavailability factor for B[a]P in humans may be estimated to be about 22% to 29%.

Analytical Capabilities. Sometimes, the reliability of bioavailability estimates is restricted by limitations of the analytical methods. Current analytical techniques may not detect all the compounds (the unaltered form and the metabolites) in the biological fluids. Also, the treated biological samples may lose or change their original form. For instance, a wide variety of methylated arsenic compounds appear in urine samples depending upon different treatment and analytical methods for different studies of arsenic exposure (Hopenhayn-Rich *et al.* 1993).

Species Difference. Bioavailability studies of xenobiotics are now rarely carried out in humans. Most bioavailability factors must be obtained from animal studies, particularly from small rodents. Extrapolation of data from animal to humans is necessary but cannot be absolutely predicted. Particularly, for some xenobiotics (chromium, arsenic, toluene, pentachlorophenol and phenol), the oral bioavailability in animals is close to estimates for humans. Dermal permeability for selected compounds in small rodents is often higher than that in humans. Unfortunely, information on the correlation of respiratory bioavailability between humans and animals is limited.

Mixtures and Vehicles. Most bioavailability studies are conducted in animals and humans by administration of a single xenobiotic. In reality, residents may be exposed to complex mixtures rather than pure chemicals. As a consequence of exposure to these mixtures, interaction effects may alter the bioavailability of each individual xenobiotic. Currently, there are no practical approaches for evaluating the effects of mixtures in bioavailaibility from estimates for each pure chemical. Direct determination of bioavailability for chemical mixtures, in reality, is necessary to better understand the health risks posed by the mixture. From a few bioavailability studies for PAH-containing mixtures, each component in the mixture has been found to exhibit a marked reduction of bioavailability in dermal exposure compared with studies of the pure PAH alone. Bioavailability will also be changed by the vehicles (soil, food, water and solvents) that carry the xenobiotic.

Mechanism of Carcinogenicity and Toxicity. Understanding the mechanisms of carcinogenicity and toxicity for xenobiotics is necessary to the development of quantitative approaches for estimating health risk. A critical determinant of carcinogenicity and toxicity is the quantity of active species that reacts at the target sites. The active species refers to either parent xenobiotics or reactive metabolites. The target sites can be the applied sites or remote sites where the active species may be delivered by the systemic circulation.

Bioavailability, by definition in pharmacokinetics, focuses on the quantity of parent xenobiotics present in the systemic circulation. When the active species are related to the parent compounds (e.g. inorganic arsenic), determination of bioavailability may provide good estimates for quantitative risk assessment. In some cases, the active species are associated with reactive metabolites (e.g. reative metabolites of benzene and benzo[a]pyrene). Therefore, the identification and quantification of the reactive metabolites or at least knowing the rate of which they are produced from the parent compound are crucial for quantitative risk assessment. For this logic, the definition of bioavailability in toxicokinetics should be extended to determine the total quantity of not only the parent compounds but also the reactive metabolites in the systemic circulation based on knowledge of the mechanism of toxicity and carcinogenicity for the selected xenobiotics.

Results from carcinogenicity tests of some xenobiotics suggest that they might induce tumors at the applied sites (such as skin and lung cancer produced by carcinogenic PAHs). In this context, data on the systemic bioavailability of such xenobiotics are not required for cancer risk assessment of these sites.

In reality, current quantitative risk assessment methods for evaluating skin cancers posed by dermal exposure to PAHs inappropriately extrapolate the dermal dose-response relationship from an oral dose-response relationship. This approach incorrectly assumes that dermal cancer risk can be evaluated on the basis of the risk posed by reactive metabolites in the systemic circulation. Notwithstanding, dermal absorption may contribute to PAH loading in the systemic circulation and cannot be neglected when evaluating PAH exposure and non-inhalation cancer risk.

Chemical Forms. The bioavailability is often affected by administration of the different chemical forms (particularly metals). Generally, various forms of a xenobiotic (elemental form, oxidation states and their salts) result in different absorption profiles. All the bioavailability values of metals in this paper (with exception for two oral bioavailability study relevant to As_2O_5 (Charbonneau *et al*. 1980, Hollins *et al*. 1979) are not related to the chemical forms known to be in the CCA-based wood preserving mixtures (CrO_3, As_2O_5, CuO). As a result, the application of these values to quantitative risk assessment for the CCA-based wood preserving mixtures islimited.

Exposure Duration and Frequency. Exposure duration and frequency in bioavailability experiments are commonly shorter compared with those of humans exposed to the environmental contaminants. This is certainty true where risk assessments attempt to evaluate cancer risks posed by exposure to chemicals over a lifetime. Some results are obtained for very short exposure periods via various routes, without knowing whether the absorption reaches a steady-state condition. In such cases, the bioavailability factors could be underestimated.

Priority Needs for Further Research

To date, there is a lack of information about the bioavailability estimates for some contaminants at the former contaminated sites in Alberta. A lack of these estimates give rise to a high level of uncertainty for quantitative risk assessment. Thus, studies of bioavailability of individual contaminants found in CCA, PCP and creosote wastes are essential for a better understanding of the health risks posed to residents near these sites.

The general population is exposed to complex mixtures of contaminants within soil, food and water rather than individual pure contaminants. In most cases, the bioavailability estimates of complex mixtures and contaminants within carriers differ from those of individual pure contaminants. Consequently, bioavailability studies of CCA-based, PCP-based and creosote-based mixtures and contaminants within carriers should be considered..

Determination of priority needs for further research and the feasibility of bioavailability study for the contaminants from the contaminated sites in Alberta depend on their physicochemical, environmental, biological and toxicological properties, primary exposure routes for the general population, experimental designs, and economic considerations (cost and time).

Respiratory bioavailability of chromium trioxide aerosols, CCA-based mixtures, hydrophobic PAHs aerosols, PAH-containing mixtures, and PCP aerosols are important and require further study. The reasons for singling out these compounds include: (1) chromium trioxide and hydrophobic PAHs aerosols are associated with an increased incidence of lung cancer in animals via inhalation; (2) carcinogenicity of chromium (VI) and PAH appears to be a localized rather than a systemic effect, so that characterization of the profiles of deposition and retention within the respiratory tract is important for these compounds; (3) PCP can produce systemic toxicity to humans via inhalation, and (4) the respiratory bioavailability of CCA-based and PAH-based mixtures may vary considerably from that of the individual components in these mixtures.

In vivo methods for studying respiratory bioavailability in animals are complex, expensive and time-consuming. Alternatively, there are some simple *in vitro* methods for determining the physicochemical and environmental properties of the xenobiotic-containing aerosols. These data may be used to predict the respiratory bioavailability of the xenobiotics. The first step is to determine the inhalable fraction (aerosol size less than 10 μm MMAD) and the particle size distribution in the air. These parameters can be used to predict the deposition of aerosols within the respiratory tract. The second step is to estimate the fractions of mechanical clearance and retention of aerosols based on available experimental data or mathematical models. The third step is to determine the dissolution fraction or rate of aerosols under conditions that simulate the respiratory environment (pH 7.4). Finally, the absorption fraction may be roughly estimated by the product of the retention fraction and dissolution fraction, by making an assumption on the absorption of all the dissolved constituents into the systemic circulation. Also, it is necessary to use the octanol/water partition coefficients of the chemicals to estimate the extent that dissolved compounds may penetrate the air-blood barrier into the systemic circulation.

Oral bioavailability studies are needed for contaminants which are likely to show large differences in bioavailability for complex mixtures compared to the individual contaminants. These contaminants consist of CCA-based mixtures, soil-bound PCP, PCP-based mixtures, soil-bound PAHs, PAH-containing mixtures and soil-bound lead at the specific contaminated sites. A study evaluating exposure via ingestion of local grown vegetables may be not important for local residents unless the local grown vegetables accumulate significant levels of the xenobiotics from the contaminated sites and comprise a significant portion of individual diets.

Measurement of biological fluids and the mass-balance technique are the most common approaches for studing oral bioavailability. Compared to the mass-balance technique, measurement of biological fluids is simple, less expensive and less time-consuming. The experimental design for measuring biological fluids must use the reference dose (intravenous dose) to account for the effects of distribution and metabolism.

Soil-bound xenobiotics that reach absorption sites must dissolve in the biological fluid from the soil matrix prior to entering the systemic circulation. Thus, determination of the dissolution fraction or rate of soil-bound xenobiotics under conditions simulating the gastrointestinal tract (e.g. pH 1-3 in the stomach and pH 7-8 in the intestine) may aid in better understanding and estimating oral bioavailability for soil-bound xenobiotics. The dissolution tests (either *in vivo* or *in vitro*) are very simple, inexpensive and less time-consuming.

Dermal bioavailability studies that focus on PAH-containing mixtures from specific contaminated sites are needed because some PAHs are skin carcinogens in animals and mixtures of them show marked differences in dermal bioavailability compared to individual pure PAHs. Both *in vivo* and *in vitro* methods could be used for these types of studies.

Table 7-1 The Potential Exposure Routes for General Population Living near Former Wood Preserving Sites

	Inhalation	Ingestion			Dermal contact	
Compound	aerosol	food	water	soil	water	soil
Chromium (VI)	+++	+	++	+	++	+
Arsenic	++	+	+++	+	+	+
Copper	++	+	+++	+	+	+
Benzene	+	+	+	+	+	+
Toluene	+	+	+	+	++	+
Pentachlorophenol	++	+++	++	++	+	+
2,4,5-trichlorophenol	++	+++	++	++	+	+
2,4,6-trichlorophenol	++	+++	++	++	+	+
2,3,4,6-tetrachlorophenol	++	+++	++	++	+	+
Hexachlorodibenzo[p]dioxin	+++	+++	+	+++	+	+++
(1)PAH vapors	+	++	++	+	++	+
(2)Hydrophobic PAHs	+++	++	++	+++	+	+++
Phenol	-	+++	+++	+	+	+
Aniline	+	++	++	+	++	+
Quinoline	-	-	-	-	-	-
Benzothiophene	-	-	-	-	-	-
Dibenzothiophene	-	-	-	-	-	-
Lead	+++	+++	+++	+++	+	+

(1): PAH vapors consist of naphthalene, 2-methylnaphthalene, acenaphthene and fluorene.

(2): Hydrophobic PAHs consist of anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene.

- : data are not available;
+: low significance;
++: moderate significance;
+++: significance

Table 7-2 The Distribution Bioavailability Factors (%) or Permeability for dermal contact for the Selected Xenobiotics from the Former Contaminated Sites in Alberta

	Inhalation		Ingestion		Dermal contact	
Compound	Human	Animal	Human	Animal	Human	Animal
Chromium (VI)	+	20-50(1) 2(2)	10(1)(3)	2(1) 10(1)(3)	$1\text{-}3.5 \times 10^3$ cm/h	1-4
Chromium (III)	+	4-10	<1-2	<1-3 10(3)	+	2
Arsenic	85-90(4)	70-80(1)(5) 50-75(2)(5) 90(1)(6)	55-100(1)(5) 100(1)(6) 25-30(2)(6)	30-100(1)(5)) 15-25(2)(5) 65-90(1)(6)	2.71×10^{-6} cm/h	+
Copper	+	+	15-70	+	+	+
Benzene	50-60(7)	+	+	90-100	<1	<1 20(8)
Toluene	30-50(7)	+	100	90-100	0.9 cm/h	+
Pentachlorophenol	90(7)	70-75	75-100	65-100	10-60(9)	25-30
2,4,5-trichlorophenol	-	-	-	-	-	-
2,4,6-trichlorophenol	-	-	-	80-95	9.9×10^4 cm/h	4.1×10^3 cm/h
2,3,4,6-tetrachlorophenol	-	-	-	-	-	-
Hexachlorodibenzo[p]dioxin	-	-	-	-	-	-
Naphthalene	+	-	+	-	-	-
2-methylnaphthalene	-	-	-	-	-	8(9)(10)
Acenaphthene	-	-	-	70	+	-
Carbazole	-	-	-	-	+	50(10- 45(9)(10)
Fluorene	-	-	-	-	+	15(9)(10)
Phenanthrene	-	-	-	100	+	80 70-80(9) 20(10) 15(9)(10)
Anthracene	-	-	-	-	+	50 55(9)
Fluoranthene	-	-	-	-	+	-
Pyrene	-	-	-	-	+	95 40-70(9) 8(10)
Chrysene	+	40-55	-	60-90	+	15(10)
Benzo[a]pyrene	+	20-80	-	10-25	25-30(9)	35-85 45-70(9) 5-16((10) 3(9)(10)

(Continued)

Compound	Inhalation		Ingestion		Dermal contact	
	Human	Animal	Human	Animal	Human	Animal
Benzo[a]anthracene	+	-	-	-	+	20(10)
Benzo[b]fluoranthene	+	-	-	-	-	20(10)
Benzo[k]fluoranthene	-	-	-	-	+	-
Benzo[g,h,i]perylene	-	-	-	-	+	-
Dibenz[a,h]anthracene	+	-	-	-	+	8-80
Indeno[1,2,3-c,d]pyrene	-	-	-	-	+	-
Phenol	60-70(7)	-	90	45-100	4-25	10-30
2,4,6-trimethylphenol	-	-	-	-	-	-
Aniline	90(7)	-	+	60	+	5
Quinoline	-	-	-	-	-	-
Benzothiophene	-	-	-	-	-	-
Dibenzothiophene	-	-	-	-	-	-
Lead	50-80(4)(5) 55-75(6)	+	40-55(11)(12) 4-13(12)(13) 30-70(3)(13) 16-20(14) 3-10(15)	+	6.3(6) 0.05(5)	6.5(6) 4.4(5)

- : Data are not available; +: Qualitative data available
(1): Data from water-soluble compounds; (2): Data from slightly ware-soluble or water-insoluble compounds; (3): In fasting status; (4): Data from the compounds deposited within the respiratory tract. The correction with the deposition fraction of the compounds needs for the application of the values; (5): Inorganic compound; (6): Organic compound; (7): vapor; (8): The applied sites are covered by a glass cover to avoid the volatilization of the compounds; (9): *in vitro* data; (01): Data from individual compounds in the mixtures; (11) Data for children; (12): In feeding status; (13): Data for adults; (14): With drinking water; (15): With diet lead

8. Conclusions

Residents living near former wood preservative sites in Alberta may be exposed to some contaminants from CCA-based, PCP-based and creosote-based mixtures via inhalation, ingestion and dermal contact. Inhalation of airborne xenobiotics and ingestion of waterborne or soil-bound xenobiotics would be the most significant exposure routes. For a few contaminants (e.g. hydrophobic PAHs), dermal contact may be an important exposure route.

Bioavailability estimates are available in the literature for some individual contaminants present in the wood preserving mixtures for specific exposure routes. These data can be used for quantitative risk assessment to improve its reliability and accuracy. These bioavailability estimates are often dependent upon physicochemical and environmental properties of xenobiotics (specific chemical form, water solubility, physical states, and inorganic or organic compound), human or animal physiological conditions (age), nutritional conditions (deficiencies of some trace-element and vitamins, and fasting or normal feeding status), and disease states (genetic diseases). Thus, the selection of bioavailability estimates for calculation of internal doses or adjustment of toxicity values must consider these variables. The characteristics of xenobiotics that are employed for bioavailability studies should be close to those that exhibit in the environment where humans may be exposed.

Bioavailability of heavy metals (chromium, arsenic, copper and lead) via some exposure routes have been extensively investigated in humans and animals. Their bioavailability is strongly affected by their chemical forms, water solubility, age of humans and animals, and nutritional conditions. The following generalizations are made:

- Chromium (VI) compounds are more readily and rapidly absorbed than chromium (III) via the main exposure routes.
- Inorganic arsenic compounds are less bioavailable in the respiratory tract and gastrointestinal tract than organic arsenic compounds.
- Organic lead compounds will more easily penetrate human or animal skin than inorganic lead compounds.

- Water soluble chromium or arsenic salts have higher bioavailability in the respiratory tract and gastrointestinal tract than the slightly water soluble or water insoluble compounds.

- Chromium (VI) in the gastric fluid (acidic environment) is readily reduced to chromium (III) absorbed less in the gastrointestinal tract.

- Children have a higher capacity for lead absorption from the gastrointestinal tract than adults.

- Fasting can enhance chromium (III) or (VI) and lead absorption from the gastrointestinal tract.

- Deficiencies of essential elements and some vitamins can increase chromium and lead bioavailability in the gastrointestinal tract.

- Daily absorption of copper (an essential element to humans) in the gastrointestinal tract is regulated based on daily requirements for human metabolism.

- Some genetic diseases may enhance lead and copper absorption in humans.

Data on bioavailability estimates for most organic compounds in the wood preserving mixtures are limited. Generally, bioavailability of these organic compounds is readily influenced by their physical states, water solubility, lipophilibility, and exposure forms (pure, mixtures or within carriers). The following generalizations are made:

- Organic vapors (benzene, toluene, PCP, phenol, aniline and PAH vapors) are highly absorbed after they are deposited within the respiratory tract.

- Pure organic liquids and solids (benzene, toluene, PCP, phenol, aniline and relatively water-soluble PAHs) are easily bioavailable in the gastrointestinal tract, and also easily penetrate the animal skin.

- Hydrophobic PAHs (e.g. B[a]P) have lower oral bioavailability than relatively water-soluble PAHs.

- Dermal bioavailability of PAH-containing mixtures or soil-bound PAHs in animals is dramatically lower than for pure compounds.

The internal dose is a refined parameter for assessing exposure and health effects. Based on its inherent properties, the application of internal dose for quantitative risk assessment has certain limitations. General considerations for using the bioavailability factor to adjust an external dose or a toxicity value include:

- Internal dose represents the quantity of a xenobiotic in the systemic circulation which may be transported to various organs and tissues to exert various toxic effects. By incorporating the bioavailability factor, the internal dose is applical for xenobiotics that produce systemic toxicity or carcinogenicity rather than those that cause local toxic effects (i.e. at the external site of application).

- Use of a bioavailability factor for the adjustment of external dose is only essential when the toxicity data (such as reference doses or slope factors) are based on internal dose.

- The route-specific bioavailability factor for each specific exposure route is useful because there may be big differences in bioavailability by different exposure routes. For instance, the respiratory bioavailability factor is only applied for assessing the internal dose via inhalation exposure.

- When assessing human health risks posed by exposure to xenobiotics in the environment, the xenobiotic characteristics modelled in the risk assessment should be similar with those from bioavailability studies and toxicity studies in order to be relevant. These characteristics include chemical form and environmental vehicles (media). For instance, when the risk assessment is conducted for general population exposure to inorganic arsenic from drinking water via ingestion, the best choice of parameters for assessing risk are the toxicity value (RfD or slope factor) and bioavailability factor from the administration of inorganic arsenic in drinking water.

- When the xenobiotic characteristics modelled in the risk assessment are not similar with those from bioavailability studies and toxicity studies, the relative bioavailability factor is a useful parameter for the adjustment of external dose. For example, when the risk assessment is conducted for general population exposure to lead from soil via ingestion but the RfD is available for lead acetate in the diet, 20% of the relative bioavailability, which is the ratio of the extent of absorption of lead in mining soil to that of lead acetate (a standard form) in the diet, may be used for adjustment.

- When toxicity values are not available for a xenobiotic for one of the three exposure routes, route-to-route extrapolation may be necessary for quantitative risk assessment. For instance, dermal toxicity data are often absent for most xenobiotics. In this case, dermal toxicity values may be extrapolated from oral or inhalation toxicity values. If the oral or inhalation values are based on external doses, these values need to be transferred into internal doses by using their bioavailability factors (oral bioavailability or respiratory bioavailability). Then, the internal dose can be related back to an external dose by consideration of dermal bioavailability.

- The distribution of reported bioavailability factors is affected by a variety of factors, such as different designs and analytical methods in the bioavailability experiments, chemical forms, types of vehicles, exposure duration and frequency, species, age, gender and nutritional conditions.

Available information on bioavailability studies for the selected xenobiotics from the former wood preserving sites is summarized in Table 8-1. A checklist for application of the bioavailability factor is showed in Figure 8-1.

For prevention of toxicity caused by exposure to environmental contaminants, there are several approaches to reduce, and, if possible, block absorption of xenobiotics into humans. Some methods, in order of decreasing values and practicality, include:

- Reduction of xenobiotic levels in the environment. Reduce exposure to xenobiotics in the environment by blocking exposure routes (e.g. capping of buried contaminants, lining of buried contaminants to protect groundwater, etc.).

- Identification of high risk groups. Individuals at high risk groups, such as young children, pregnant women, the elderly, heavy smokers, people with nutritional deficiencies and some genetic diseases, are more vulnerable to exposure to environmental contaminants. Thus, correction of nutritional deficiencies, hygienic education for young children, reduction of smoking, treatment of relevant diseases are important approaches for reducing absorption of certain xenobiotics from the human body.

- Use of antidotes. Using an antidotes to block absorption or other steps relevant to toxicity can be a effective method for treating acute poisoning. This method is not feasible for general large populations who are chronically exposed to environmental contaminants at relatively low levels. In some cases, a specific antidotes may be applied for very small groups of people at high risk to reduce absorption of specific xenobiotics from the human body.
- Reduction of xenobiotic body burden. After absorption, xenobiotics are distributed to various organs and tissues. One way to minimize toxic effects to humans is to reduce the body burden. Chelation therapy for metal intoxication provides a means for reducing the body burden of the toxic metals. Chelators can bind free metal ions to remove these toxic metals from the body pool. The most common chelating agents include dimercaprol (BAL) for lead and arsenic, EDTA for lead, and penicillamine for copper and lead. However, commonly, chelation therapy is only suitable for acute metal intoxication.

Table 8-1 Available information on the bioavailability factors for the Selected Xenobiotics from the Former Contaminated Sites in Alberta

	Inhalation		Ingestion		Dermal contact	
Compound	Human	Animal	Human	Animal	Human	Animal
Chromium (VI)	-	✓	✓	✓	-	✓
Chromium (III)	–	✓	✓	✓	-	✓
Arsenic (inorganic)	✓	✓	✓	✓	-	-
Arsenic (organic)	-	✓	✓	✓	-	-
Copper	-	-	✓	-	-	-
Benzene	✓	-	-	✓	✓	✓
Toluene	✓	-	✓	✓	-	-
Pentachlorophenol	✓	✓	✓	✓	✓	✓
2,4,5-trichlorophenol	-	-	-	-	-	-
2,4,6-trichlorophenol	-	-	-	✓	✓	✓
2,3,4,6-tetrachlorophenol	-	-	-	-	-	-
Hexachlorodibenzo[p]dioxin	-	-	-	-	-	-
Naphthalene	-	-	-	-	-	-
2-methylnaphthalene	-	-	-	-	-	✓
Acenaphthene	-	-	-	✓	-	-
Carbazole	-	-	-	-	-	✓
Fluorene	-	-	-	-	-	✓
Phenanthrene	-	-	-	✓	-	✓
Anthracene	-	-	-	-	-	✓
Fluoranthene	-	-	-	-	-	-
Pyrene	-	-	-	-	-	✓
Chrysene	-	✓	-	✓	-	✓
Benzo[a]pyrene	-	✓	-	✓	✓	✓
Benzo[a]anthracene	-	-	-	-	-	✓
Benzo[b]fluoranthene	-	-	-	-	-	✓
Benzo[k]fluoranthene	-	-	-	-	-	-
Benzo[g,h,i]perylene	-	-	-	-	-	-
Dibenz[a,h]anthracene	-	-	-	-	-	✓
Indeno[1,2,3-c,d]pyrene	-	-	-	-	-	-

(Continued)

Compound	Inhalation		Ingestion		Dermal contact	
	Human	Animal	Human	Animal	Human	Animal
Phenol	✓	-	✓	✓	✓	✓
2,4,6-trimethylphenol	-	-	-	-	-	-
Aniline	✓	-	-	✓	-	✓
Quinoline	-	-	-	-	-	-
Benzothiophene	-	-	-	-	-	-
Dibenzothiophene	-	-	-	-	-	-
Lead (inorganic)	✓	-	✓	-	✓	✓
Lead (organic)	✓	-	-	-	✓	✓

✓: bioavailability factor estimates available

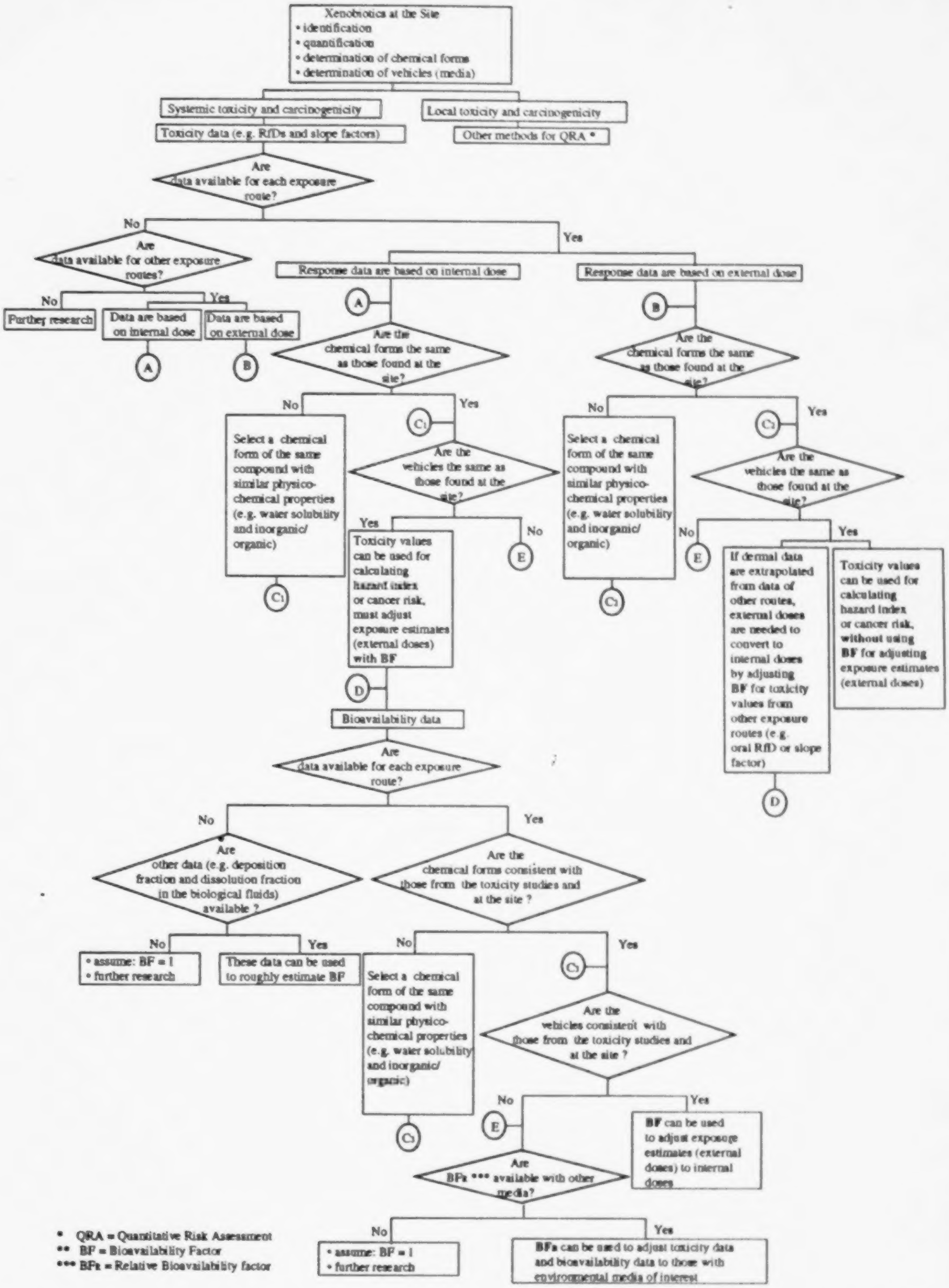
Xenobiotics at the Site
• identification
• quantification
• determination of chemical forms
• determination of vehicles (media)
Systemic toxicity and carcinogenicity
Local toxicity and carcinogenicity
Toxicity data (e.g. RfDs and slope factors)
Other methods for QRA *
Are data available for each exposure route?
No
Yes
Are data available for other exposure routes?
No
Yes
Further research
Data are based on internal dose
Data are based on external dose
A
B
Response data are based on internal dose
Response data are based on external dose
A
B
Are the chemical forms the same as those found at the site?
Are the chemical forms the same as those found at the site?
No
Yes
No
Yes
Select a chemical form of the same compound with similar physico-chemical properties (e.g. water solubility and inorganic/organic)
C1
C1
Are the vehicles the same as those found at the site?
Yes
No
Toxicity values can be used for calculating hazard index or cancer risk, must adjust exposure estimates (external doses) with BF
E
Select a chemical form of the same compound with similar physico-chemical properties (e.g. water solubility and inorganic/organic)
C2
C2
Are the vehicles the same as those found at the site?
No
Yes
E
If dermal data are extrapolated from data of other routes, external doses are needed to convert to internal doses by adjusting BF for toxicity values from other exposure routes (e.g. oral RfD or slope factor)
Toxicity values can be used for calculating hazard index or cancer risk, without using BF for adjusting exposure estimates (external doses)
D
D
Bioavailability data
Are data available for each exposure route?
No
Yes
Are other data (e.g. deposition fraction and dissolution fraction in the biological fluids) available ?
No
Yes
• assume: BF = 1
• further research
These data can be used to roughly estimate BF
Are the chemical forms consistent with those from the toxicity studies and at the site ?
No
Yes
Select a chemical form of the same compound with similar physico-chemical properties (e.g. water solubility and inorganic/organic)
C3
C3
Are the vehicles consistent with those from the toxicity studies and at the site ?
No
Yes
E
BF can be used to adjust exposure estimates (external doses) to internal doses
Are BFR *** available with other media?
No
Yes
• assume: BF = 1
• further research
BFR can be used to adjust toxicity data and bioavailability data to those with environmental media of interest
* QRA = Quantitative Risk Assessment
** BF = Bioavailability Factor
*** BFR = Relative Bioavailability factor

Figure 8-1 Checklist for application of bioavailability factor in quantitative risk assessment

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